ENDOCRINOLOGY

THE BULLETIN OF
THE ASSOCIATION FOR THE STUDY
OF INTERNAL SECRETIONS

VOLUME 25 AUGUST, 1939 NUMBER 2

THE NEUROHYPOPHYSIS AND WATER EXCHANGE IN THE MONKEY[1]

H. W. MAGOUN, C. FISHER AND S. W. RANSON
From the Institute of Neurology
Northwestern University Medical School
CHICAGO, ILLINOIS

IN A RECENT monograph, Fisher, Ingram and Ranson (1) have presented a large amount of evidence which indicates that the elaboration of an antidiuretic hormone by the neurohypophysis is dependent upon the innervation of this gland via the infundibulum by a large tract of nerve fibers whose most important component arises from the supraoptic nuclei of the hypothalamus. In both the cat and monkey hypothalamic lesions so placed as to interrupt the supraoptico-hypophyseal tract were followed by retrograde degeneration of the supraoptic nuclei and by an alteration of cell structure and marked shrinkage of the neurohypophysis (i.e., infundibular process, infundibular stem and median eminence), together with the loss of its secretory activity as determined by assay methods, with the resulting development of a marked polyuria and polydipsia characteristic of diabetes insipidus. In the cat the same symptoms have been shown to follow complete extirpation of the neurohypophysis or sections of the infundibular stalk which were high enough to deprive most of the neurohypophysis of its innervation.

It has been emphasized that the supraoptico-hypophyseal system forms an anatomical and functional unit and that injury to any part of it is reflected by the presence of degenerative changes in the other parts. When, however, the tracts are interrupted in the hypothalamus, not only the infundibular process, but also the median eminence and infundibular stem are deprived of their nerve fibers. This is important because the last 2 structures are histologically similar to the infundibular process and in all probability all 3 parts are concerned in the elaboration of the antidiuretic principle. We believe that the failure of many investigators to produce experimental diabetes insipidus by stalk section or attempted extirpation of the neurohypophysis has been in large part due to their failure to make the line of incision high enough, in both instances the median eminence being left intact. These conclusions

[1] Aided by grants from the Rockefeller Foundation and the Committee on Research in Endocrinology of the National Research Council.

have been confirmed by the positive results which have been obtained in the cat after high section of the stalk or total extirpation of the neurohypophysis.

In the monkey, however, only negative results have been reported following extirpation or transection experiments. Thus Mahoney and Sheehan (2) and Foerster, Gagel and Mahoney (3) have recently reported that cutting or putting a silver clip on the infundibular stalk in the monkey does not result in diabetes insipidus, and on the basis of these results and a study of the supraoptic nuclei in such cases (4), have concluded that supraoptico-hypophyseal connections are very few in number and of little functional significance in the monkey, and that in this form diabetes insipidus results from the impairment of some intrinsically hypothalamic mechanism. Since the supraoptico-hypophyseal connections, contrary to the findings of these workers, have recently been shown to be as numerous in the monkey as in other animals (5, 15) it seemed highly probable that appropriate interruption of these connections in this form would lead to diabetes insipidus. The present report deals with this phase of the problem.

Because of the emphasis which we have placed on such an apparently minute structure as the median eminence, an attempt has been made to determine what proportion in terms of volume it forms of the total normal neurohypophysis. In addition the volumes of the degenerated parts of the neurohypophysis distal to the line of transection and volumes of the portions of the gland proximal to this line were determined in the operated animals. These measurements were then correlated with the presence, absence and degree of polyuria shown by the different animals.

METHODS

The animals were operated on with aseptic precautions under deep nembutal anesthesia. A vertical skin incision on the right side from the zygoma to the top of the head exposed the temporal muscle, which was split vertically and retracted anteriorly and posteriorly. A large area of calvarium was removed with rongeurs and the dura was sectioned parallel to the defect, but was left intact ventrally. A broad retractor was inserted under the temporal lobe, elevating it and exposing a field bordered by the optic and oculomotor nerves and traversed by the internal carotid artery. After excess cerebrospinal fluid was removed, a tiny knife with a ball tip and rounded anterior edge was inserted behind the internal carotid artery so as to retract it anteriorly. The posterior cutting edge of the knife was thus brought in front of the infundibular stalk, which was transected by a backward movement of the knife. The dura, temporal muscle and subcutaneous tissue were sutured in layers and the skin closed with clips. The animals were operated on in the morning, being kept on a heating pad, and had usually recovered from the anesthesia to the extent of sitting up and eating by late afternoon.

Of 17 animals so prepared, 15 were studied for 9 to 12 weeks after operation, and 1 was killed 3 weeks, and 1, 6 weeks postoperatively. Attention was directed particularly to water exchange as measured by urine output. Urine was collected in metabolism cages, the drinking water being supplied from reservoir bottles to avoid spilling. In some animals urine outputs were recorded for a few days at a time with longer intervals when no records were made, in others continuous daily records for the entire period of survival were obtained. The weights of the animals were re-

corded at operation and when killed, and they were observed for signs of adiposity. In several cases the blood sugar reactions to insulin were investigated and are reported elsewhere (6).

The animals were killed by bleeding under ether or nembutal, and the vessels of the head were washed out with normal saline solution and perfused with 10% formalin, so that the brain and hypophysis were fixed in situ. The base of the cranium was carefully dissected away leaving the dura and all the intradural contents, including those of the sella, intact.

In each case a single block including the hypothalamus and the contents of the sella, together with the dura enclosing them, was removed, imbedded in paraffin and sectioned serially at 10μ, in the transverse plane. Every fourth section through the series was stained with cresyl violet and every twentieth section with Bodian's silver method. Alternate fourth sections through the region of transection were stained by van Gieson's method. Similar preparations from 4 normal, unoperated monkeys were available, 2 of which were prepared in the identical manner with the operated cases.

In order to determine accurately the level of the stalk sections midsagittal reconstructions of the brain and hypophysis were made with a projection apparatus on millimeter-ruled graph paper at a magnification of 20 times, and checked with the microscope. Using the same method in each case, the volume of the neurohypophysis was determined by drawing the outlines of sections, taken at intervals of 80 to 120μ (constant in each specimen) through the entire gland and projected at a magnification of 60 times. The area of the outlines was then calculated with a planimeter, corrected for magnification and multiplied by the distance between the sections. The volume determined was less than that of the true volume at autopsy because of the shrinkage involved in preparing the material for microscopical examination. It may be that some variation in shrinkage occurred in the different cases, though with the exception of 2 of the 4 normal cases all of the material was prepared in the same manner. As will be pointed out in the presentation of the data, however, the differences in volumes to which significance has been attached are obviously very much greater than could have occurred through variations in the amount of shrinkage.

Diabetes Insipidus

Preoperative data. It is difficult to give any absolute figure as to the daily urine output of normal monkeys of a given weight for the output of each animal varied from day to day, and the average daily outputs over a period of 1 to 2 weeks also showed considerable variation in different animals. The quantity may be expressed in cubic centimeters per kilogram of body weight. In 8 monkeys weighing between 3.3 and 3.7 kg., the low extreme was as little as 15 cc./kg. (sp. gr. 1.032) with average outputs slightly under 30 cc./kg. (1.023). The high extremes were in the neighborhood of 90 cc./kg. (1.010) with average outputs somewhat over 60 cc./kg. (1.015). For the purposes of the present study 90 cc./kg. has been adopted as the upper limit of normal.

Following operation the characteristic series of alterations associated with experimental diabetes insipidus (1) occurred, namely a transient phase, an interphase and a permanent phase.

Transient phase. A transient phase of polyuria was present after operation in 9 cases and was inferred to have been present in 2 other animals which

were not in metabolism cages, but which exhibited a postoperative polydipsia. One animal with transection of the infundibular stem did not show a transient polyuria. Two other animals showed questionable transients in that the specfic gravities were lower than normal though the urine outputs were not excessively high. No observations were made in 3 cases. In the group with transient polyuria were animals with transection of the median eminence or infundibular stem or incomplete section of the stem.

In all but one instance the greatest output in this phase occurred during the night following operation; in the exceptional case it occurred during the succeeding day. The maximum output ranged between 115 and 300 cc./kg; the specific gravity of the urine was low and ranged between 1.005 and 1.008. The observations on urine output of 3 animals still under anesthesia on the afternoon of the day of operation are worthy of note. They had been without water for the 4-hour period spent in preparing and operating on them, and did not recover from the anesthesia sufficiently to move and obtain water for an additional period of 1, 3 and 4 hours respectively. During this postoperative period, 1 animal passed 55 cc. of urine (1.005) in 1 hour, the second passed 100 cc. (1.008) in 3 hours and the third passed 140 cc. (1.003) in 4 hours. These observations clearly indicate that the transient polyuria can appear independently of the ingestion of water.

The animal with the greatest transient polyuria, of 300 cc./kg., had a transection of the median eminence and later developed a marked permanent polyuria. However, another such case showed a transient polyuria of only 115 cc./kg. and a third animal with a section of the stalk below the median eminence, whose urine output ultimately became normal, had a transient polyuria of 255 cc./kg. The severity of the transient polyuria cannot, therefore, be specifically correlated with the site of injury or the subsequent alteration in urine output in these cases.

Interphase. In the animals in which it was present the transient polyuria abated during the second to fourth postoperative day and a period of several days then followed during which time the urine output was not above normal limits. In the middle portion of this interphase the animals exhibited a tendency toward a low urine output with a high specific gravity, and in 5 cases this tendency amounted to a definite oliguria. On the sixth postoperative day 3 animals had respectively outputs of 6 cc./kg. (1.050), 10 cc./kg. (1.042) and 13 cc./kg. (1.043). On the seventh postoperative day 2 others had outputs of 12 cc./kg. (1.047) and 9 cc./kg. (1.038). This oliguria could not be attributed to nutritional disturbances for the animals were eating well.

Permanent phase. The onset of the permanent phase was observed in 11 cases and occurred between the ninth and the sixteenth postoperative day. Following the appearance of the permanent phase the animals could be separated into rather well defined groups.

Marked permanent polyuria. This condition developed in 4 animals and continued without improvement until they were killed. The smallest of these animals, weighing 2.5 kg., had a polyuria averaging 410 cc./kg. (1.006) for the entire period of the permanent phase, and 408 cc./kg. for the last month of survival, the lowest daily output amounting to 296 cc./kg. and the

highest to 600 cc./kg. The second animal weighing 3.4 kg. had a polyuria averaging 318 cc./kg. for the entire period of the permanent phase, and 307 cc./kg. for the last month of survival, the lowest daily output being 244 cc./kg. and the highest 400 cc./kg. The polyuria of the third animal, weighing 3.5 kg. averaged 388 cc./kg. for the entire period and 369 cc./kg. for the last month, the lowest daily output being 257 cc./kg. and the highest 437 cc./kg. The largest animal, weighing 4.2 kg., had a polyuria averaging 580 cc./kg. (1.005) for the entire period of the permanent phase, and 582 cc./kg. for the last month of survival, the smallest 24 hour output amounting to 490 cc./kg. and the largest to 660 cc./kg.

Mild permanent polyuria. This condition developed in 3 animals. The first, weighing 3 kg., had an average output of 110 cc./kg. for the entire period of the permanent phase. The highest daily output of 177 cc./kg. was reached 9 days after the onset of the permanent phase, the polyuria reaching a peak at this time and then declining somewhat. The average output for the last month of survival was 96 cc./kg., and during the last month the daily output was below 90 cc./kg., the upper limits of normal, on 10 occasions the lowest being 50 cc./kg. The highest daily output in the last month was 133 cc./kg. The second animal, weighing 3.7 kg., had an average output of 122 cc./kg. for the entire period of the permanent phase. The highest output of 211 cc./kg. was reached on the third and again on the seventh day after the onset of the permanent phase, the polyuria reaching a peak at this time and then diminishing. The average output for the last month was 114 cc./kg., and during the last month the output was below 90 cc./kg. on 8 occasions, the lowest being 49 cc./kg. The highest output in the last month was 189 cc./kg. The third animal, weighing 3.3 kg., had an average output of 127 cc./kg. for the entire period of the permanent phase, and the average output for the last month was 119 cc./kg. The urine output was below 90 cc./kg. on only 1 occasion during the last month when it fell to 85 cc./kg. The highest output of 176 cc./kg. was reached during the last month, there being no clear-cut peak following the appearance of the permanent phase.

Brief secondary polyuria. This resembled the permanent polyuria in character and time of onset, developed in 4 cases and lasted for 4, 5, 8 and 12 days. The urine outputs then returned to within normal limits and with isolated exceptions remained normal for the remainder of the survival time. During the brief polyuria the peak outputs amounted to 103, 160, 217 and 262 cc./kg. A fifth case, not placed in the metabolism cage until the twentieth postoperative day, had outputs within normal limits most of the time, but occasionally showed high outputs, the highest being 125 cc./kg.

No permanent polyuria. This condition was observed in 5 animals, though 2 of these were not under observation during the time of onset of the permanent phase and may or may not have shown a brief secondary polyuria.

Summary. Of 17 monkeys with experimental section of the infundibular stalk, 4 developed a marked permanent polyuria, 3 had a mild permanent polyuria, 5 showed a brief secondary polyuria with return to normal, and in 5 no permanent phase was observed. In the chronic state, therefore, 7 monkeys were polyuric and 10 had urine outputs within the normal range.

The point of interest now to be determined is whether any correlation exists between this symptomatology and the amount of damage to the neurohypophysis.

Location of the Lesions

The neurohypophysis of the normal monkey can be divided into a median eminence, infundibular stem and infundibular process (fig. 1A). The median eminence lies in immediate contact with the tuberal region of the hypothalamus and forms the floor of the infundibular recess of the third ventricle. Distally it tapers into the infundibular stem which still further distally enlarges into the infundibular process. As can be seen in figure 1A, the infundibular stalk in the monkey is so exposed that experimental section can be accomplished either through the median eminence or through the proximal portion of the infundibular stem below it.

Marked permanent polyuria. The lesions in these cases were all located in the median eminence (fig. 1B; 2A, B, C). The smallest section (fig. 1B) was

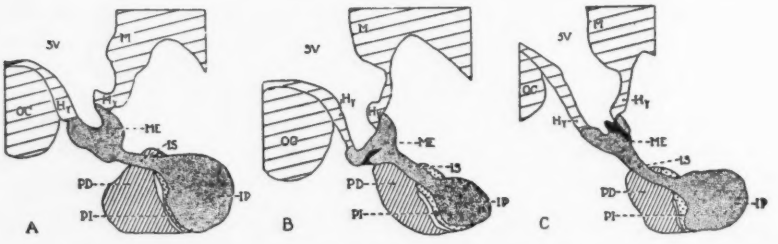


Fig. 1. MIDSAGITTAL RECONSTRUCTIONS OF THE HYPOTHALAMUS AND HYPOPHYSIS. A, a normal monkey; B, monkey with section of the anterior half of the median eminence; C, monkey with section of the posterior half of the median eminence. The sections are shown in solid black.

Abbreviations for all figures are as follows: AC, anterior commissure; Hy, hypothalamus; IP, infundibular process; IS, infundibular stem; M, mammillary region; ME, median eminence; OC, optic chiasma; PD, pars distalis; PI, pars intermedia; 3 V, third ventricle.

confined to the anterior half of the median eminence and there was no injury to the hypothalamus. In the case shown in figure 2A, the median eminence was completely interrupted through its lower part, without injury to the hypothalamus. In the case shown in figure 2B, the section completely interrupted the median eminence, and also involved the vascular supply to the tuberal region of the hypothalamus on the side opposite that approached with the resulting unilateral softening projected in cross-hatching on the wall of the ventricle in the figure. In the case shown in figure 2C, the median eminence was completely interrupted and the hypothalamus was uninjured except for slight scattered gliosis and connective tissue proliferation at the junction of median eminence and tuber. The case shown in figure 1B had during the last month of its survival a polyuria averaging 307 cc./kg.; figure 2A, 582 cc./kg.; figure 2B, 408 cc./kg.; and figure 2C, 369 cc./kg.

Mild polyuria. In the case averaging 96 cc./kg. during the last month of its survival the section involved the infundibular stem below the median

eminence, and what appeared to be the proximal end of the stem above the section was greatly enlarged. The connective tissue scar did not form a complete band across the section, an area of stem about 40μ wide being free and it was difficult to decide whether this minute area had been spared, but since the infundibular process contained no nerve fibers, the section was assumed to be complete. In the case averaging 114 cc./kg., during the last month the junction between median eminence and stem was completely severed, and the vascular supply on the side opposite that of approach was evidently involved for the median eminence on that side was much reduced in size and a unilateral softening extended into the tuberal region of the hypothalamus similar to that shown in figure 2B. In the case averaging 119 cc./kg. during the last month, no clear-cut section was present, but there was marked connective tissue proliferation in the proximal end of the infundibular stem and to a lesser extent in the median eminence above it, and it is concluded that this region was severely traumatized though not actually sectioned.

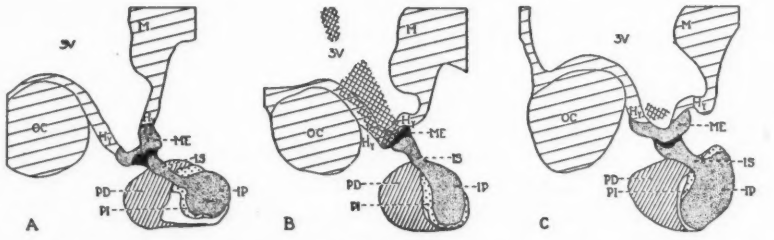


Fig. 2. MIDSAGITTAL RECONSTRUCTIONS OF THE HYPOTHALAMUS AND HYPOPHYSIS OF 3 MONKEYS WITH COMPLETE SECTIONS OF THE MEDIAN EMINENCE. The sections are shown in solid black. The extent of concomitant injury to the hypothalamus in the cases shown in B and C has been projected in cross-hatching on the wall of the third ventricle. Abbreviations as in figure 1.

Brief secondary polyuria. The lesions in these cases all consisted of sections of the infundibular stem just below the median eminence. Two of these were unquestionably complete and are shown in figure 3A and B. In a third case the connective tissue scar did not form a complete band across the level of section, a minute area along one edge being free, but no nerve fibers were present in the infundibular process and the section was assumed to be complete. The 2 other cases had only partical sections and nerve fibers could be observed in the infundibular process, though this was markedly shrunken.

Polyuria absent. The lesions in these cases consisted in 4 instances of complete sections of the infundibular stem just below the median eminence and 1 case is illustrated in figure 3C.

Unlike these 4, the fifth case in which no polyuria was observed had a section confined to the posterior half of the median eminence, shown in figure 1C. An unsuccessful attempt to reach the stalk anterior to the internal carotid artery during operation in this animal resulted in a softening of the lateral aspect of the optic chiasma which extended into the medial portion of the supraoptic nucleus on the side of approach.

Summary of the relation of the location of the lesion to the degree of the

polyuria. These cases may be divided into a group with lesions located in the median eminence and a second group with lesions located in the infundibular stem just below it.

Complete transection of the median eminence or section of its anterior half alone was associated with marked and enduring permanent polyuria. The polyuria in these cases cannot be attributed to associated operative injury to the hypothalamus for such injury was absent in 2 cases, unilateral in a third and only minimal in a fourth. Section of the posterior half alone of the median eminence did not result in permanent polyuria.

Total or incomplete transection of the infundibular stem below the median eminence resulted either in mild and enduring permanent polyuria, brief secondary polyuria with return to normal or no permanent polyuria at all. The unequivocal information which is provided by a number of cases of

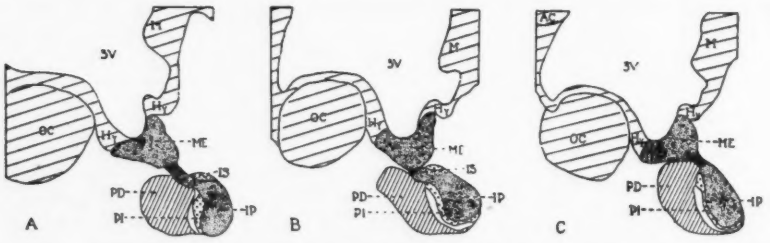


Fig. 3. MIDSAGITTAL RECONSTRUCTIONS OF THE HYPOTHALAMUS AND HYPOPHYSIS OF 3 MONKEYS WITH COMPLETE SECTIONS OF THE INFUNDIBULAR STEM. The sections are shown in solid black. Abbreviations as in figure. 1.

this group is that urine outputs may be within the normal range following complete section of the proximal end of the infundibular stem with resulting isolation of this structure and the infundibular process from connections with the hypothalamus. It does not necessarily follow that the antidiuretic mechanism is entirely normal in such cases for Fisher, Ingram and Ranson (1) have shown that cats in which the neurohypophysis had been similarly reduced and which showed no impairment under ordinary conditions exhibited a marked abnormality when subjected to the stress of thyroid or salt feeding.

The fact that transection of the median eminence results in marked polyuria, while transection of the infundibular stem below it is compatible with the maintenance of a normal urine output, can be variously interpreted. It might be suggested that the median eminence alone, and still more specifically only its anterior half, was concerned with the antidiuretic activity of the neurohypophysis, but we are not familiar with any histological or pharmacological evidence which suggests such a precise localization of this function. A more reasonable explanation would appear to be that all parts of the neurohypophysis are concerned in antidiuretic activity, and that, as in other organs, there is a large factor of safety, so that only a fraction of the gland is sufficient to preserve physiological conditions. Since the activity of the neuro-

hypophysis is dependent upon its innervation from the supraoptic nuclei of the hypothalamus, such functional remnants must of necessity be proximal to the section in order that this innervation may be preserved. It seemed desirable to see to what extent a quantitative study of the volumes of the parts of the neurohypophysis in the operated and normal monkeys would support this explanation.

Neurohypophysis

Volumetric data on the neurohypophysis. These data which are presented in table 1 include the measurements from 4 normal monkeys and from 11 operated animals with complete transections either through the median eminence or through the infundibular stem below it. None of the animals were fully grown but ranged in body weight from 2.3 to 5.2 kg. The neurohypophysis undoubtedly increases in size over the age period which these monkeys represent, but no information on this subject is available, and for want of any better method, the volumes will be considered both absolutely and in terms of cubic millimeters per kg. of body weight.

The average absolute volume of the entire neurohypophysis of 4 normal monkeys as determined from sections cut in paraffin was 12.93 cu. mm.: of this the median eminence made up 13%, the infundibular stem 3%, and the infundibular process 84%. The average volume of the neurohypophysis per kg. of body weight was 5.0 cu. mm.

In 3 cases with marked polyuria, 0.28, 0.25 and 0.32 cu. mm./kg. of neurohypophysis remained proximal to the section and connected with he hypothalamus. These quantities represent 5, 5 and 6% of the total normal volume and were associated with polyurias of 582, 408 and 369 cc./kg., respectively.

Two cases with mild polyuria had 0.34 and 0.75 cu. mm./kg. of neurohypophysis proximal to the section and connected with the hypothalamus. These volumes represent 7 and 15% of the normal and were associated with polyurias of 114 and 96 cc./kg. respectively.

Six cases with normal urine outputs had between 0.50 and 0.70 cu. mm./kg. of neurohypophysis proximal to the section and connected with the hypothalamus, these volumes representing between 10 and 14% of the normal. The urine outputs ranged between 83 and 23 cc./kg.

If one case is compared with another inconsistencies can be noted which argue against any very precise correlation between a given volume of neurohypophysis and a specific urine output. If, however, the results are considered more generally, it is apparent that the group with marked polyuria had much smaller volumes of neurohypophysis remaining in connection with the hypothalamus than did the group with normal urine outputs. In terms of cubic millimeters per kg. of body weight, the neurohypophysis connected with the hypothalamus in the 3 cases with marked polyuria averaged 5% of normal, while in the 6 cases with normal outputs, it averaged 12% of the normal. If the absolute volumes are considered the same difference is seen, for the neurohypophysis connected with the hypothalamus in the cases of marked polyuria then averaged 7% of the normal, while in the cases with

normal outputs it averaged 16% of the normal. The cases with normal urine outputs therefore possessed from 128 to 140% more neurohypophyseal tissue than did the cases with marked polyuria, a difference which is obviously too great to be explained by any conceivable variations in shrinkage, etc.

It is not difficult to understand that a deficiency symptom may result following the loss of from 93 to 95% of a gland, but in deciding whether it is reasonable to assume that from 12 to 16% (1/6 to 1/8) may be sufficient to prevent deficiency symptoms, the following references to data on other organs are of interest. Ingle, Hales and Haslerud (7) have shown that rats with only about 20% of the normal amount of adrenal tissue will survive indefinitely without presenting any overt evidence of adrenal insufficiency. Partial removal, even to the extent of seven-eighths of the pancreas does not produce diabetes mellitus (8). If even a small part of one testicle—not more than one-sixteenth—is left, castration changes do not occur, though the portion which is left generally undergoes hypertrophy (8). After reduction of the mass of hepatic tissue in the dog to less than 20% of the normal, this small part will maintain all of the functions of the entire liver over a period of several months (9). It is only when renal function, as measured by the urea clearance, has fallen to 20% of the normal that the concentration of urea in the blood becomes definitely elevated (10). With proper diet the normal weight of the dog may be maintained after removal of all of the stomach, 6 feet of small intestine and two-thirds of the pancreas (11). Such evidence of the tremendous physiological reserve possessed by various organs of the body, including endocrine glands, indicates that the maintenance of a normal urine output with only 1/6 to 1/8 of the normal amount of the neurohypophysis in connection with the hypothalamus, is not a physiological incongruity.

Hypertrophy of the neurohypophysis remaining in connection with the hypothalamus. This has recently been reported by Swann (12) after incomplete post-hypophysectomy in the rat. In the present investigation, data on this subject are furnished by the 6 animals with normal urine outputs (table 1), all of which had complete sections of the infundibular stem just below the median eminence, leaving this structure in connection with the hypothalamus. A comparison of the volume of the median eminence in these cases (fig. 3A, B, C) with that of the normal animals (fig. 1A) should indicate whether or not hypertrophy had occurred. In the normal the median eminence averaged 1.65 cu. mm. or 0.68 cu. mm./kg. and constituted 13% of the total neurohypophysis. Considered with reference to body weight, the median eminence in the 6 operated animals averaged 0.61 cu. mm./kg. or 12% of the total neurohypophysis, and the close correspondence to normal might be taken as indicating that no hypertrophy had occurred. If, however, the absolute measurements are considered the median eminence in the operated cases averaged 2.15 cu. mm., as against 1.65 cu. mm. for the normal, suggesting that a hypertrophy of 30% had occurred. But these monkeys were older and larger. It may be said that the present results are inconclusive as to whether hypertrophy occurred or not since no data are available as to the relative normal rates of growth of the body and neurohypophysis. It is clear,

however, that the neurohypophyseal tissue remaining in connection with the hypothalamus did not undergo atrophy. This is surprising for one would expect atrophy in a median eminence which had lost something like two-thirds of the fibers which ordinarily pass through it on their way to the more distal portions of the neurohypophysis. It is clear that because of the integrity of its innervation from the supraoptico-hypophyseal tracts, the median eminence has retained its normal size or perhaps increased it. If this innervation is interrupted by placing lesions in the hypothalamus the median eminence undergoes atrophy along with the infundibular stem and process. This is shown by the results of the measurements in monkey M5 (previously described by Fisher, Ingram and Ranson, 1), whose median eminence had a volume of 0.87 cu. mm., which is about one-half the normal volume. The absence of atrophy of the median eminence as a result of the degeneration of the supraoptico-hypophyseal tract, resulting from section of the infundibular stem, may perhaps indicate that a hypertrophy of the other elements of the neurohypophysis has taken place.

TABLE 1. VOLUMES OF PARTS OF THE NEUROHYPOPHYSIS IN NORMAL MONKEYS AND IN MONKEYS WITH COMPLETE SECTION OF THE MEDIAN EMINENCE (GROUP A) OR OF THE INFUNDIBULAR STEM (GROUP B)

	Body weight	Vol. of median eminence	Vol. of infundibular stem	Vol. of infundibular process	Total vol. of neurohypophysis connected with the hypothalamus		Av. urine output during last month of survival
	kg.	*cu. mm.*	*cu. mm.*	*cu. mm.*	*cu. mm.*	*cu. mm. per kg.*	*cc. per kg.*
Normal controls	?	1.38	.34	12.34	14.06	?	
	2.3	1.45	.40	11.44	13.29	5.7	
	2.7	1.71	.40	9.72	11.83	4.4	
	2.6	2.07	.44	10.06	12.57	4.8	
Group A. Section of median eminence	4.2	1.19	.21	3.18	1.19	.28	582
	2.5	.69	.18	2.06	.62	.25	408
	3.5	1.19	.24	4.53	1.12	.32	369
Group B. Section of infundibular stem	3.7	1.26	.17	6.14	1.26	.34[1]	114
	3.0	1.24	1.07	2.12	2.26	.75	96
	5.2	2.62	.18	3.20	2.62	.50	83
	2.1	1.22	.14	2.27	1.22	.58	76[2]
	3.4	2.25	.16	2.44	2.25	.66	61
	3.0	1.91	.21	3.04	1.91	.63	36
	3.2	2.24	—	2.91	2.24	.70	36
	4.2	2.67	—	3.13	2.67	.64	23

[1] This monkey had some unilateral damage to the median eminence above the line of section.
[2] Killed during third postoperative week, urine outputs from 16 to 23 postoperative days.

Atrophy of those portions of the neurohypophysis whose connection with the hypothalamus were severed. This was evident in both the infundibular stem and the infundibular process. The latter, being the larger structure, may be compared in the operated (fig. 2A, B, C; 3A, B, C) and normal (fig. 1A) animals. Taking absolute measurements, the volume of the infundibular process of 11 operated animals (table 1) amounted to an average of only 31% of the normal volume, and if considered with reference to body weight, the

volume in the operated cases was only about one-quarter of the normal. The infundibular process was not directly affected by the lesions in any of these operated animals, nor could the lesions have interfered with the blood supply to this region which, as Wislocki (13, 14) has shown, enters and leaves the infundibular process at its posterior aspect. This atrophy is the same as that described by Fisher, Ingram and Ranson (1) following hypothalamic lesions interrupting the nerve supply to the neurohypophysis.

It is of interest to compare the amount of atrophy in the infundibular process in the cases of this series in which the anterior half alone and the posterior half alone of the median eminence was sectioned (fig. 1B and C). As shown elsewhere (15) these 2 sections interrupted widely different amounts of the supraoptico-hypophyseal tract, for in the case with the posterior section only about 30% of the cells of the supraoptic nucleus were lost, while in the case with the anterior section 80% of the supraoptic cells had degenerated. In the case with section of the posterior half of the median eminence, the infundibular process had a volume of 7.05 cu. mm. (body weight 4.5 kg.), which is 70% of the normal volume in absolute measurement and 38% in terms of cu. mm. per kg. of body weight. In the case with section of the anterior half of the median eminence, the infundibular process had a volume of 3.61 cu. mm. (body weight 3.4 kg.), which is 35% of the average normal value in absolute measurements and 24% in terms of body weight. This value closely approximates those following complete stalk section. It would appear that interruption of connections from the supraoptic nuclei was a potent, though probably not the only factor leading to atrophy of the neurohypophysis.

Other Parts of the Hypophysis

Anterior lobe. This was not affected in any of the 5 cases with section through the median eminence nor was it injured in 4 cases with section of the infundibular stem. In 8 animals with section of the stem a softening was present in the vicinity of the stem and a wing-shaped defect extended downward and backward into both sides of the pars anterior. In the affected areas the glandular cells were entirely removed, even as early as 3 weeks postoperatively, and were replaced by connective tissue. In 5 of these cases the softened area was quite restricted, in 2 it was somewhat more extensive, and in 1 case it involved the entire rostrodorsal portion of the anterior lobe. This was the only case to show a sensitivity to insulin (6). No correlation could be detected between the anterior lobe injury and the urine output in the various cases. The softening in the pars distalis undoubtedly resulted from interruption of the afferent blood supply which Wislocki and King (16) and Wislocki (13, 14) have shown to approach the anterior lobe in the walls of and immediately around the stalk. The absence of softening in some cases and its variable extent in others was probably dependent upon the degree of interruption of afferent vessels at operation and the rapidity with which collateral channels became established.

Pars tuberalis. The pars tuberalis which forms a saddle over the back and

sides of the upper portion of the median eminence and surrounds its lower portion and the proximal part of the infundibular stem, was of necessity injured in all these experiments. Though no volumetric measurements were made of this structure it was evident, however, that large proportions were left intact in every case. No difference could be observed in the parts of the pars tuberalis above and below complete sections. In our opinion no correlation can be made between the symptomatology and injury to the pars tuberalis in the different animals.

Adiposity

No marked alterations in weight occurred in any of the animals. Of the 15 kept for from 9 to 12 weeks after operation, 10 animals retained their initial weights within 0.1 kg. One animal lost 0.2 kg. Four animals gained 0.3, 0.4, 0.7 and 0.9 kg. None exhibited a polyphagia. None showed pathological collections of fat at autopsy. The gastrointestinal tracts were normal at autopsy in all cases but 1. The exception was an animal which had shown no gastrointestinal symptoms for 8 weeks after operation but which then developed a mucous, bloody diarrhea. On killing it was found to be suffering from colitis.

That pathological adiposity may develop within the period of survival of the animals of this series has been shown in other experiments by Ranson, Fisher and Ingram (17) who observed the development of marked polyphagia and pathological adiposity in a monkey 3 weeks after lesions had been placed in the hypothalamus. Ten weeks after operation this animal had gained 2.8 kg. and doubled its weight. The case was complicated by the presence of a diabetes mellitus, however, and no conclusions regarding the etiology of the pathological obesity were drawn. The present experiments indicate that pathological adiposity does not result from the complete interruption of direct nervous connections as well as other possible channels of communication between the hypothalamus and hypophysis up to 12 weeks after operation.

SUMMARY

In a study of the importance of the hypothalamic innervation of the neurohypophysis for the regulation of water exchange in the monkey, a series of animals were subjected to subtemporal section of the infundibular stalk and observed for periods up to 12 weeks after operation.

In the different animals marked permanent polyuria, mild permanent polyuria, brief secondary polyuria or normal urine outputs were encountered.

A study of serial sections of the hypophysis and overlying hypothalamus prepared in every case showed that the animals with marked polyuria had complete transections of the median eminence or section of its anterior half alone, while the animals with the less severe symptoms or with normal urine outputs had sections of the infundibular stem below the median eminence or through the posterior half of the median eminence.

Volumetric determinations showed that in the cases with marked polyuria an average of only 5 to 7% of the total volume of the normal neurohypoph-

ysis remained proximal to the section and connected with the hypothalamus. In the cases with normal urine outputs, an average of 12 to 16% of the total volume of the neurohypophysis remained connected with the hypothalamus proximal to the section. Transection of the median eminence or of the infundibular stem regularly caused marked atrophy of the infundibular process.

The maintenance of a normal urine output with the preservation of only 1/6 to 1/8 of the neurohypophysis is comparable to the demonstrations of physiological reserve reported for other organs of the body, including endocrine glands, by various workers.

It seems likely that the negative results encountered by other investigators following low stalk sections in the monkey are to be explained by the sizeable amount of neurohypophysis left proximal to the section, connected with the hypothalamus and innervated by the supraoptic nuclei.

The authors are indebted to Miss Mary Ranson for the preparation of the drawings.

REFERENCES

1. Fisher, C., W. R. Ingram and S. W. Ranson: Diabetes insipidus and the neuro-hormonal control of water balance: A contribution to the structure and function of the hypothalamico-hypophyseal system. Edwards Brothers, Ann Arbor, 1938.
2. Mahoney, W., and D. Sheehan: *Brain* 59: 61. 1936.
3. Foerster, O., O. Gagel and W. Mahoney: *Verhandl. d. deutsch. Gesellsch. f. inn. Med., Kong.* 49: 165. 1937.
4. Gagel, O., and W. Mahoney: *Ztschr. f. d. ges. Neurol. u. Psychiat.* 156: 594. 1936.
5. Rasmussen, A. T.: *Endocrinology* 23: 263. 1938.
6. Brobeck, J. R.: To be published. 1939.
7. Ingle, D. J., W. M. Hales and G. M. Haslerud: *Am. J. Physiol.* 113: 200. 1935.
8. Sharpey-Schafer, E.: The endocrine organs, an introduction to the study of internal secretion, part II. 2nd edition. Longmans, Green and Co., Ltd., London. 1926.
9. Bollman, J. L., and F. C. Mann: *Ergebn. d. Physiol.* 38: 445. 1936.
10. Smith, H. W.: The physiology of the kidney. Oxford University Press, New York. 1937.
11. Ivy, A. C.: Personal communication. 1939.
12. Swann, H. G.: *Proc. Soc. Exper. Biol. & Med.* 39: 255. 1938.
13. Wislocki, G. B.: The Pituitary Gland, Assn. for res. nerv. and ment. dis. 17: 48. 1938.
14. Wislocki, G. B.: *Anat. Rec.* 72: 137. 1938.
15. Magoun, H. W., and S. W. Ranson: *Anat. Rec.* in press. 1939.
16. Wislocki, G. B., and L. S. King: *Am. J. Anat.* 58: 421. 1936.
17. Ranson, S. W., C. Fisher and W. R. Ingram: *Endocrinology* 23: 175. 1938.

RELATION BETWEEN THE GROWTH PROMOTING EFFECTS OF THE PITUITARY AND THE THYROID HORMONE[1]

HERBERT M. EVANS, MIRIAM E. SIMPSON AND RICHARD I. PENCHARZ

From the Institute of Experimental Biology, University of California
BERKELEY, CALIFORNIA

ALTHOUGH it is clearly established that hypophysectomy in young animals leads to abrupt and complete cessation of growth and that anterior pituitary implants or extracts restore this growth, considerable confusion exists as to the participation of the thyroid in these phenomena. That dwarfism follows the complete thyroidectomy of young animals has also been securely established, a remarkable dwarfism following thyroidectomy of the new born (1, 2). Thyroid-parathyroid medication of such animals led to the restoration of normal growth. A partial analogy with such animals is furnished by the rare genetic strain of silver dwarf mice investigated by Smith and MacDowell (3, 4), MacDowell and Laanes (5), Bates, Laanes and Riddle (6), Kemp and Marx (7), and others, for such animals can be induced to grow with thyroid substance. They also respond to growth promoting extracts of the pituitary as do thyroidectomized rats (8). Experiments with silver dwarf mice and hypophysectomized pigeons have led some investigators (9) to declare that the stimulation of growth by pituitary extracts is at least partially due to the thyrotropic hormone. Salmon has stated that pituitary implants did not influence the remarkable growth stasis produced by parathyro-thyroidectomy performed shortly after birth, although if a thyroid remnant was present the same treatment produced growth. The last mentioned work was done with anterior pituitary implants, only two daily having been employed in the highest dosage. Smith (10) and the present authors have shown that if sufficient anterior pituitary extract be given, normal growth can be produced in thyroidectomized-hypophysectomized animals. The Salmon work may therefore be looked upon as showing merely that an amount of pituitary growth promoting substance inadequate for the promotion of growth in the absence of the thyroid is adequate to promote growth when thyroid tissue is present. Confirming and extending our own previous work and that of Smith, the present experiments demonstrate, it is believed, that a synergism exists between the thyroid hormone and the growth promoting substance or substances in anterior pituitary extract—a synergism also shown in cases of the established absence of the

[1] Aided by grants from the Board of Research of the University of California and the Rockefeller Foundation, New York City.

thyroid gland so that the thyrotropic hormone of the pituitary is not implicated.

A large group of female rats 35 to 45 days of age was parathyro-thyroidectomized and after an average period of 3 months evidence of the completeness of the operation was sought in the degree of depression of the B.M.R. and degree of growth retardation. Approximately 50 individuals were found which gave this evidence of the completeness of the thyroidectomy. Furthermore, in some cases all structures in the thyroid region (trachea, esophagus, surrounding muscle, etc.) were removed and examined in serial section for fragments of the thyroid. No thyroid fragments were found in animals in which less than 130 l. $O_2/mm.^2/24$ hours were consumed (normal 150). The B.M.R. of the animals chosen for experimental purposes averaged 30% below normal. Complete growth stasis occurred in the group as can be seen in figure 1. Some increase in weight occurred during the first 10 days postoperative (12 gm., normal 30 gm.), then ceased until very late after thyroidectomy, when some increase in weight occurred due to adiposity, not skeletal growth.

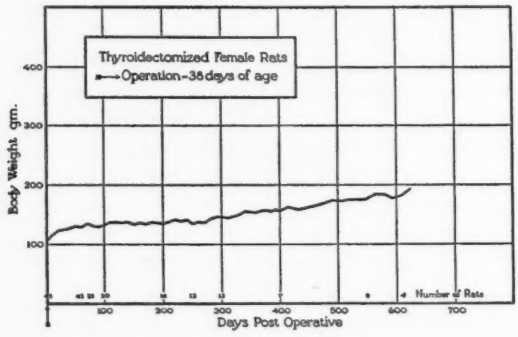


Fig. 1

The animals were divided into 4 groups of 5 or 6 each and treated as follows: *a*), uninjected controls; *b*), injected daily with 0.005 mg. of crystalline thyroxin—this dose having been found adequate for restoration of the B.M.R. to normality; *c*), injected daily with 1 cc. of a standardized alkaline extract of bovine anterior hypophysis; *d*), injected with the last mentioned extract with the addition of thyroxin, the thyroid and pituitary substances being injected at the levels employed in groups *b* and *c*. Similar numbers of normal female rats, as nearly as possible of the same body weight as those thyroidectomized, were injected with thyroxin, anterior pituitary extract, and the combination of these two substances. Figure 2, and tables 1 and 2, show that after 3 months the largest giants produced in the experiments resulted from the injection of the combination of anterior pituitary extract and thyroxin and no differences existed between thyroidectomized rats so treated and normal ones. Normals injected with the pituitary extract alone

were very large, but significantly smaller than the doubly treated animals. Thyroidectomized animals treated with pituitary extract were next in size

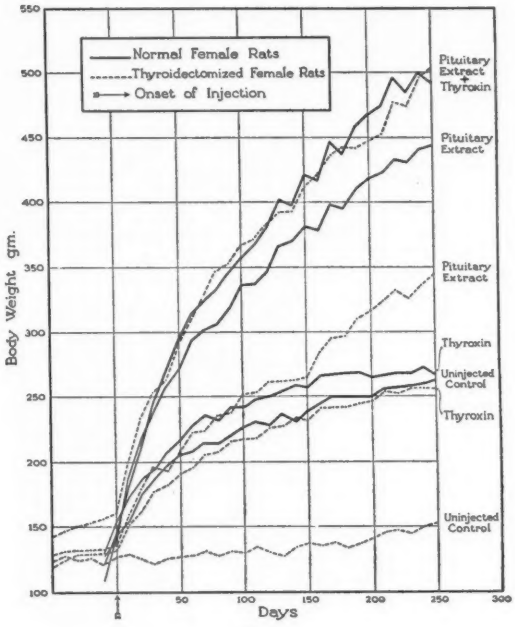


Fig. 2

and were appreciably larger than any normal control. Thyroidectomized animals treated with thyroxin alone had conferred upon them a normal

TABLE 1. FINAL BODY WEIGHTS OF FEMALE RATS

	Controls	Thyroxin	Growth hormone	Growth hormone +thyroxin
	gm.	gm.	gm.	gm.
Normal	263	268	445	492
Thyroidectomized	133	256	345	503
Thyroidectomized-hypophysectomized	136	155	250	400

TABLE 2. FINAL BODY LENGTHS OF FEMALE RATS

	Controls	Thyroxin	Growth hormone	Growth hormone +thyroxin
	cm.	cm.	cm.	cm.
Normal	40.8	40.7	44.0	46.7
Thyroidectomized	32.6	38.4	37.8	44.9
Thyroidectomized-hypophysectomized	30.0	31.9	37.1	46.0

growth rate, the untreated thyroidectomized controls remaining dwarfed. The treatment of normal animals with thyroxin did not modify their growth.

These experiments seem to show: *a*), growth promotion in excess of normality secured by anterior pituitary extracts is not conditional on the presence of the thyroid, but *b*), is greater when the thyroid is present, and *c*), in thyroidectomized animals becomes equally great if thyroxin is concordantly administered.

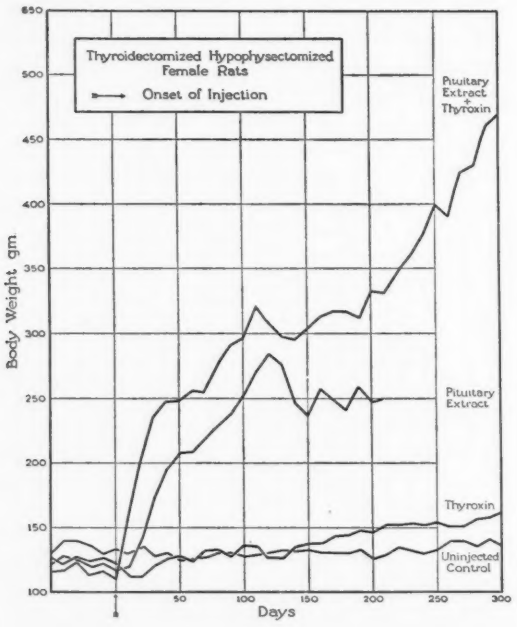


Fig. 3

As the restoration of normal growth produced by the administration of thyroxin to thyroidectomized animals was interpretable as due to stimulation of the pituitary, a number of thyroidectomized animals were also hypophysectomized,[2] the usual precautions being taken to establish proof of the completeness of the operation. One day after hypophysectomy the rats were divided into groups[3] which received the same 3 types of treatment as had been given normal and thyroidectomized rats. In contrast to the effect in thyroidectomized rats, thyroxin did not alter the dwarfism of the doubly operated rats (fig. 3). The pituitary extract, however, conferred the same growth on the hypophysectomized-thyroidectomized animals as upon thyroidectomized animals, and in the one remaining survivor the simultaneous

[2] The usual search of the sella was made at autopsy to establish completeness of hypophysectomy, no results from animals possessing fragments being included.

[3] The groups were smaller due to the high mortality of the doubly operated animals.

treatment with thyroxin and pituitary extract ultimately led to the same maximal growth as had been seen in the thyroidectomized animals similarly treated. The experiments with the doubly operated animals show that the stimulation of the growth of thyroidectomized animals secured with thyroxin can occur only in the presence of the pituitary, the growth effect of which on the peripheral tissues is synergically improved, the synergism between growth promoting extracts and thyroxin being equally demonstrable with animals from which both glands have been removed.

Organ Changes

Adrenals. The adrenals in the thyroidectomized groups were small, though not as small as those characterizing hypophysectomized rats or thy-

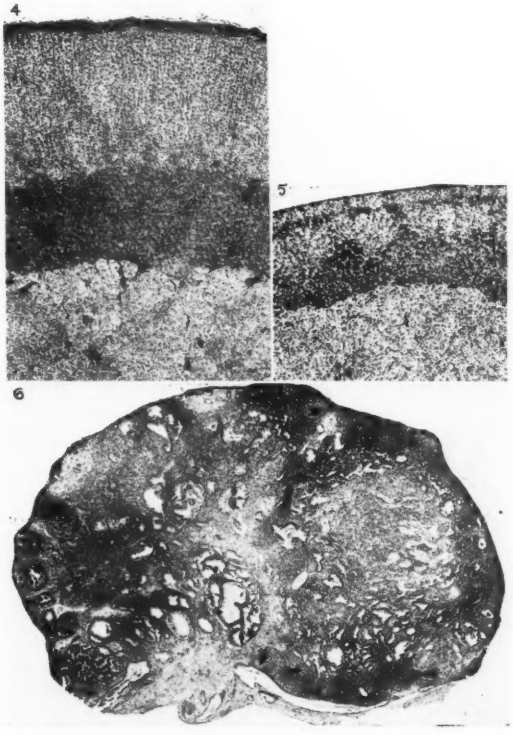


Fig. 4. ADRENAL, NORMAL RAT, AGE 305 DAYS. ×35. Fig. 5. ADRENAL, RAT THYROIDECTOMIZED AT 35 DAYS OF AGE; 365 DAYS OF AGE AT AUTOPSY. ×35. Fig. 6. OVARY, RAT THYROIDECTOMIZED AT 35 DAYS OF AGE; GROWTH HORMONE INJECTED FROM DAY 113 TO AUTOPSY AT DAY 365. ×17.

roidectomized-hypophysectomized rats, 20 mg. vs. 9 mg., (fig. 4, 5 and table 3, 4). The adrenal cortex of the thyroidectomized rats was greatly reduced in thickness, due to the reduced width of both fasciculata and reticularis;

TABLE 3. AVERAGE WEIGHTS OF ADRENALS OF FEMALE RATS

	Controls	Thyroxin	Growth hormone	Growth hormone +thyroxin
	mg.	*mg.*	*mg.*	*mg.*
Normal	72	58	57	75
Thyroidectomized	20	61	47	59
Thyroidectomized-hypophysectomized	9	10	22	26

TABLE 4. ADRENAL MEASUREMENTS (EXPRESSED IN CENTIMETERS, MAGNIFIED ×50)

	Greatest dimension of gland	Width[1] of cortex	Greatest dimension of medulla
Normal uninjected	23.5	5.4	12.9
Thyroidectomized uninjected	14.4	2.6	8.9

[1] All values are average values from 3 rats. The cortical width was determined in each animal from the average of 4 measurements taken at equidistant points.

the glomerulosa was actually increased; the medulla was also reduced, the cells being smaller and the nuclei pyknotic. In doubly operated rats, the medulla was like that in thyroidectomized rats, but the cortical changes were even more extreme. Thyroxin did not repair the medullary (or cortical)

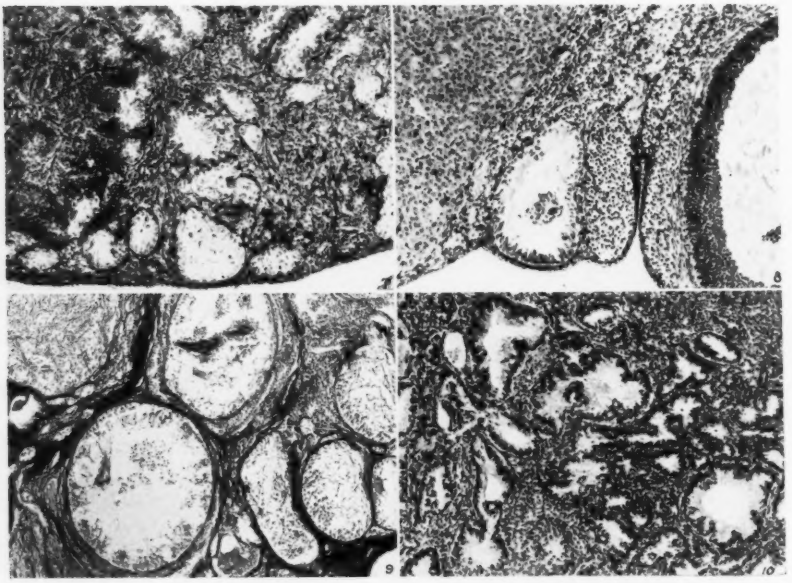


Fig. 7. OVARY, RAT THYROIDECTOMIZED AT 59 DAYS OF AGE; 792 DAYS OF AGE AT AUTOPSY. ×73. Fig. 8. OVARY, RAT THYROIDECTOMIZED AT 34 DAYS OF AGE; 651 DAYS OF AGE AT AUTOPSY. ×73. Fig. 9. OVARY, RAT THYROIDECTOMIZED AT 35 DAYS OF AGE; GROWTH HORMONE INJECTED FROM DAY 113 TO AUTOPSY AT DAY 365. ×73. Fig. 10. OVARY, RAT THYROIDECTOMIZED AT 35 DAYS OF AGE; GROWTH HORMONE INJECTED FROM DAY 113 TO AUTOPSY AT DAY 365. ×73.

changes in thyroidectomized-hypophysectomized rats, though it caused both medullary and cortical repair when thyroidectomy only had been performed, indicating action through the pituitary. Growth promoting hormone caused almost complete adrenal repair in the thyroidectomized animals and partial repair in the doubly operated rats.

Genital Tract. Gonadal changes were indicated in the thyroidectomized rats even before autopsy. The vaginal cycles remained normal for only a short period, the cycles then became prolonged, were frequently abortive in nature, or ceased entirely for long periods. During such prolonged diestrus the vaginal lips became very thin and pale and the orifice was reduced to pinpoint size. Animals that had been in this condition for long periods (a year postoperative) might have an occasional cycle.[4] The ovaries were found at autopsy to be subnormal in size, weighing between 14 and 45 (average 26) mg., while the ovaries of normal controls weighed 71 mg. The size of the largest ovaries was due to cyst formation. Small numbers of healthy or degenerating corpora lutea were usually present; if follicles were present they were usually only small or medium in size. The thing of unusual interest in these ovaries was, however, the abundance of hilum tubules, and anovular follicles, which were so abundant at times that they occupied all the space usually taken by the interstitial tissue and sometimes reached the tunica albuginea (fig. 6; fig. 7, 8). These structures, probably derived from the 'male' hilar tubules, also appear to develop in part from atretic follicles. In thyroidectomized animals treated with thyroxin (alone, or with growth hormone) these tubules were not present, but growth hormone alone did not prevent their appearance (fig. 9, 10). The doubly operated (thyroidectomized-hypophysectomized) rats did not show this peculiar transformation of the ovary. They contained remnants of old corpora lutea and almost no follicles (a few small follicles without antra were present), the interstitial tissue being deficient.

CONCLUSIONS

Growth promotion in excess of normal (gigantism) secured by anterior pituitary extracts is not dependent on the presence of the thyroid but is greater when the thyroid is present.

The growth promotion of thyroidectomized animals or thyroidectomized-hypophysectomized animals in excess of normal (gigantism) secured by anterior pituitary extracts is also maximal only if thyroxin is concordantly administered.

Thyroxin, which promotes the growth of thyroidectomized animals, does not have this effect when administered to thyroidectomized-hypophysectomized animals.

Characteristic ovarian changes are found in thyroidectomy of long standing. These consist essentially in the substitution of medullary for cortical

[4] In estimating reports in the literature to the effect that thyroidectomy in the rat does not disturb the cycle, it should be remembered that complete removal of the thyroid in the rat is not easy and that workers have frequently not established the completeness of the operation.

activity, a growth of the so-called medullary or hilum tubules occurring; in extreme cases the latter structures dominate the ovary, ovarian follicles, corpora lutea and normal interstitial tissue being absent.

The administration of thyroxin to such thyroidectomized females prevents this development of the hilum tubules, but anterior pituitary extracts do not have this effect.

REFERENCES

1. SALMON, T. N.: *Proc. Soc. Exper. Biol. & Med.* 35: 489. 1936.
2. SALMON, T. N.: *Endocrinology* 23: 446. 1938.
3. SMITH, P. E., AND E. C. MACDOWELL: *Anat. Rec.* 46: 249. 1930.
4. SMITH, P. E., AND E. C. MACDOWELL: *Anat. Rec.* 50: 85. 1931.
5. MACDOWELL, E. C., AND T. LAANES: *Carnegie Inst. Wash. Yr. Bk.* 31: 47. 1932.
6. BATES, R. W., T. LAANES AND O. RIDDLE: *Proc. Soc. Exper. Biol. & Med.* 33: 446. 1935.
7. KEMP, T., AND L. MARX: *Acta Path. et Microbiol. Scandinav.* 13: 512. 1936.
8. FLOWER, C. F., AND H. M. EVANS: *Anat. Rec.* 29: 383. 1924.
9. BATES, R. W., O. RIDDLE, E. L. LAHR AND J. P. SCHOOLEY: *Am. J. Physiol.* 119: 603. 1937.
10. SMITH, P. E.: *Proc. Soc. Exper. Biol. & Med.* 30: 1252. 1933.

THE CORTINOMIMETIC ACTIVITY OF VARIOUS STEROLS[1]

J. A. WELLS AND R. R. GREENE
From the Department of Physiology and Pharmacology
Northwestern University Medical School
CHICAGO, ILLINOIS

VARIOUS OBSERVERS have shown that life is maintained in adrenalectomized animals which have functional corpora lutea (1, 2, 3), and it has recently been shown that artificially produced corpora lutea tend to increase the life span of the adrenalectomized rat (4, 5). This would suggest that the corpus luteum produces a substance or substances which are cortinomimetic in the adrenalectomized animal. Many of the known sex hormones chemically resemble the life-maintaining substances from the adrenal cortex, namely corticosterone (6), dehydrocorticosterone (7, 8) and desoxycorticosterone (9). The latter compound is progesterone with the addition of a hydroxyl group on the 21-carbon atom.

Progesterone, recently isolated from the adrenal cortex (10), has been shown by Gaunt, Nelson and Loomis (11) to be capable of bringing about a gain in weight in the adrenalectomized immature rat. These authors have obtained a survival during treatment of 9 in a group of 19 animals with dosages of 1 to 2 mg. This same phenomenon has been observed in another mammal, the ferret (12). The repsonse has been obtained with relatively lower dosages.

In a preliminary report (13) we have shown that crystalline progesterone is capable of maintaining 9 out of 10 treated rats for the duration of the treatment, in doses of 4 mg. per day. The animals were maintained in good condition and showed a progressive increase in weight. Cessation of treatment resulted in a loss of weight and subsequent death from adrenal insufficiency. Fischer and Engle have reported similar findings (5).

Due to the findings of Gaunt, Nelson and Loomis (11), who obtained a cortinomimetic effect with lower dosages than those used by us, we have determined whether a smaller daily dose of crystalline progesterone is capable of producing a significant effect in our animals. In conjunction with this investigation a number of other crystalline hormones, which might be suspected of having cortinomimetic activity, have been studied. They were selected for trial either on the basis of chemical similarity, or on the basis of their previously demonstrated 'sodium-retaining activity' in the dog. With reference to the sodium-retaining activity it has been shown that certain sex hormones (estradiol, progesterone, estrone, pregnandiol, testo-

[1] Supported in part by a grant from the Josiah Macy Jr. Foundation.

sterone and testosterone propionate) cause sodium retention, similar to the cortical hormones, in the normal dog (14).

METHODS

Male rats weighing 40 to 50 gm. were bilaterally adrenalectomized. Early in the experiment 4 animals weighing less than 40 gm. were used (lowest 36 gm.). Following adrenalectomy, treated and control animals were kept under identical conditions with regard to room temperature and diet, which consisted of Purina Fox Chow and water ad libitum. In a very few instances (small animals) the diet was supplemented on the first few days with a few grams of powdered milk. All of the animals were weighed daily for the duration of the experiment. The various hormones were administered subcutaneously in oil solution or suspension. The injections were made at the time of weighing to avoid excessive handling of the animals.

The capsule was removed from the superior pole of the kidney along with the adrenal, since this is the site at which most regeneration of the remaining adrenal tissue takes place. However, in approximately 15% of the adrenalectomized animals, life and growth were maintained for periods in excess of 3 weeks. At autopsy, adrenal tissue was present in these animals. Macroscopic findings were confirmed in every case by histologic examinations. These animals are not included in the various tables. In some instances, animals died in less than 24 hours after operation. These animals have not been included in the tables as we feel that death in these instances was due to causes other than adrenal insufficiency.

RESULTS

Progesterone. Twenty-five immature adrenalectomized male rats have been treated with varying dosages (1 to 4 mg. per day) of crystalline progesterone dissolved in oil. It may be said with certainty that 4 mg. of crystalline progesterone per day will maintain the life of immature adrenalectomized male rats (40 to 50 gm.), and also bring about a progressive increase in body weight. These animals (table 1a) showed an average weight gain of 65.8%. Ninety per cent lived for the duration of the treatment period, 15 days. Death did not occur in these animals until 3 days (average) following the cessation of treatment. However, one of the animals died on the 12th day of treatment after showing a progressive gain in weight during this period. Autopsy demonstrated no cause for death.

Three milligrams of crystalline progesterone produced 14.3% gain in weight and maintained 57% of the animals for the complete 15-day period. Two milligrams of progesterone per day, produced a 22.1% gain in weight and 1 mg. a 12.6% gain in weight. Only the 2 mg. dose was capable of maintaining any of the animals (2 out of 5 treated animals) for the complete period of 15 days. It would appear that 1 mg. of crystalline progesterone per day, while capable of producing an increase in weight in immature animals, is not capable of life maintenance for an appreciable length of time in the rats of our colony.

Pregnandiol and allopregnandiol. The natural reduction products of progesterone, pregnandiol and allopregnandiol were tested for cortinomimetic activity. These substances were assayed in oil suspension. As is shown in table 1a pregnandiol in doses of 5.0, 7.5 and 10.0 mg. displayed no corti-

nomimetic activity. Subcutaneous crystalline implants of this substance on the day of adrenalectomy (5.0 to 23.0 mg.) were also ineffective. Allopregnandiol was given in dosages of 5.0 to 15.0 mg. per day. A slight increase in survival time and also a slight weight increment was obtained with the highest dosage, but these changes were not considered significant.

TABLE 1. CORTINOMIMETIC EFFECT OF VARIOUS STEROLS

Treatment	No. animals	Daily dose in mg.	% Surv. treat.	Ave. wt. at oper.	Ave. post-oper. surv. time	% change in wt. last day of life	% change in wt. in mock oper. controls[1]
Controls	27	0	0	42.4	5.44	− 3.8	29.5
a							
	10	4.0	90.0	42.4	18.00	+65.8	107.0
Progesterone	7	3.0	57.0	47.5	12.40	+14.3	73.5
	5	2.0	20.0	43.4	9.60	+22.1	60.0
	5	1.0	0	46.0	10.40	+12.6	67.5
Pregnandiol	4	10.0	0	45.0	4.25	−14.4	24.3
	5	7.5	0	44.6	4.80	−11.2	24.8
	8	5.0	0	42.0	6.37	+6.0	40.0
Preg. Cryst.	7	5–23.0	0	43.2	4.00	− 3.9	24.0
Allopreg.	5	15.0	0	40.8	7.80	+ 3.4	49.2
	8	7.5	0	40.5	6.20	− 2.8	39.0
	2	5.0	0	39.0	2.00	− 2.6	14.0
b							
Test. Prop.	3	1.25–5.	0	38.7	3.30	+ 3.5	20.0
Test. Pell.	4	2.0–5.	0	44.0	3.25	− 1.86	21.0
Andro. dione.	6	2.0–8.	0	45.2	5.66	+ 2.2	30.0
Dehydroand.	2	3.0	0	42.0	4.00	− 6.0	24.0
c							
Est.	2	2.0	0	49.0	5.00	−12.2	25.0
Dif.	3	4.0	0	40.6	4.30	−12.3	24.3
Dieth. Stilb.	7	4.0	0	47.6	4.44	−12.1	24.4
Triphen. Ethyl.	5	4.0	0	46.8	4.40	−12.8	24.4
Dihyd. diphen.	4	5.0	0	41.7	3.20	− 8.8	20.0
d							
Desoxycholic	4	5.0	0	46.5	4.50	− 4.5	24.5
Dehydrocholic	4	5.0	0	42.0	4.25	− 5.9	24.3

[1] This column represents the percentage change in weight in 15 mock operated controls on the day corresponding to the last day of survival in the treated groups.

Androgens. Various androgens have been shown to have a sodium retaining action (12) and for this reason were also tested for cortinomimetic power in the adrenalectomized rat. As can be seen in table 1b no effect was obtained with the dosages used. Testosterone propionate (1.25 to 5.0 mg. daily) was ineffective. Testosterone pellets (2.0 to 5.0 mg.) were implanted on the day of adrenalectomy, but no results were obtained. Androstenedione (2.0 to 8.0 mg. daily) and dehydroandrosterone (3.0 mg. daily) were likewise ineffective.

Estrogens. Estradiol dipropionate, in doses of 2.0 to 4.0 mg. per day (table 1c) has been tested. The results were negative. Several of the synthetic

estrogens have also been tested with the idea in mind that these substances might have the sodium retaining action of the naturally occurring estrogens without their toxic effect in adrenalectomized animals (12, 15).

No cortinomimetic action was shown when 4:4′-dihydroxy diphenyl was given in oil suspension (5.0 mg. daily). Results were also negative with diethyl stilbestrol (4.0 mg. daily) and triphenyl ethylene (4.0 mg. daily).

Bile acids. Desoxycholic acid, one of the bile acids, has a slight chemical similarity to progesterone and corticosterone, and for this reason was tested for cortinomimetic activity. No such activity was shown in dosages of 5.0 mg. per day. Dehydrocholic acid, another of these compounds also proved ineffective in doses of 5.0 mg. per day.

We are well aware that the number of animals used in most of these groups is small, but the large doses used should give an indication of qualitative response. We feel that the only real criterion for cortinomimetic activity should be a maintenance of life and therefore, when large doses of the material are used without obtaining such evidence, it is reasonably safe to conclude that if there is any activity present it is very slight.

SUMMARY

Progesterone has been shown to be cortinomimetic and in dosages of 4.0 mg. daily is adequate for the maintenance of adrenalectomized immature rats. Pregnandiol, allopregnandiol, testosterone proprionate, androstenedione, dehydroandrosterone, estradiol dipropionate, diethyl stilbestrol, triphenyl ethylene, 4:4′-dihydroxy diphenyl, desoxycholic acid and dehydrocholic acid in the dosages used, showed no cortinomimetic activity.

We wish to thank Dr. Ernst Oppenheimer, Ciba Pharmaceutical Products, Inc., for the progesterone, testosterone, testosterone proprionate, androstenedione, dehydroandrosterone; Dr. Oliver Kamm, Parke, Davis & Co. for the pregnandiol and allopregnandiol; and Dr. J. A. Morrell, E. R. Squibb & Sons for the triphenyl ethylene, and diethylstilbestrol.

REFERENCES

1. Swingle, W. W., W. M. Parkins, A. R. Taylor, H. W. Hays, and J. A. Morrell: *Am. J. Physiol.* 119: 675. 1937.
2. Rogoff, J. M., and G. N. Stewart: *Am. J. Physiol.* 86: 20. 1928.
3. Rogoff, J. M., and G. N. Stewart: *Am. J. Physiol.* 88: 162. 1929.
4. D'Amour, M. C., and F. E. D'Amour: *Proc. Soc. Exper. Biol. & Med.* 40: 417. 1939.
5. Fischer, A., and M. Engle: *Lancet* 1: 354. 1939.
6. Reichstein, T.: *Helv. Chim. Acta* 19: 1107. 1936.
7. Reichstein, T.: *Helv. Chim. Acta* 20: 953. 1937.
8. Mason, H. L., W. M. Haehn, B. F. McKenzie and E. C. Kendall: *J. Biol. Chem.* 120: 719. 1937.
9. Steiger, M., and T. Reichstein: *Nature* 139: 925. 1937.
10. Beall, O., and T. Reichstein: *Nature* 142: 479. 1938
11. Gaunt, R., W. O. Nelson, and E. Loomis: *Proc. Soc. Exper. Biol. & Med.* 39: 319. 1938.
12. Gaunt, R., and H. Hayes: *Science* 88: 576. 1938.
13. Greene, R. R., J. A. Wells, and A. C. Ivy: *Proc. Soc. Exper. Biol. & Med.* 40: 83. 1939.
14. Thorn, G. W., and G. A. Harrop: *Science*, 86: 40. 1937.
15. D'Amour, F. E., and D. Funk: *J. Pharmacol. & Exper. Therap.* 62: 307. 1938.

METABOLISM STUDIES IN THE ALBINO RAT

CARBOHYDRATE STUDIES AFTER HYPOPHYSECTOMY[1]

R. A. PHILLIPS[2] AND PHOEBE D. ROBB

From the Laboratories of the Collis P. Huntington Memorial Hospital of Harvard University

BOSTON, MASSACHUSETTS

HOUSSAY, Mazzocco and Rietti (1) reported a decrease in liver glycogen in frogs following hypophysectomy. Because of the widespread use of the albino rat in metabolism studies it was deemed advisable to study the carbohydrate metabolism in the rat following hypophysectomy.

METHODS

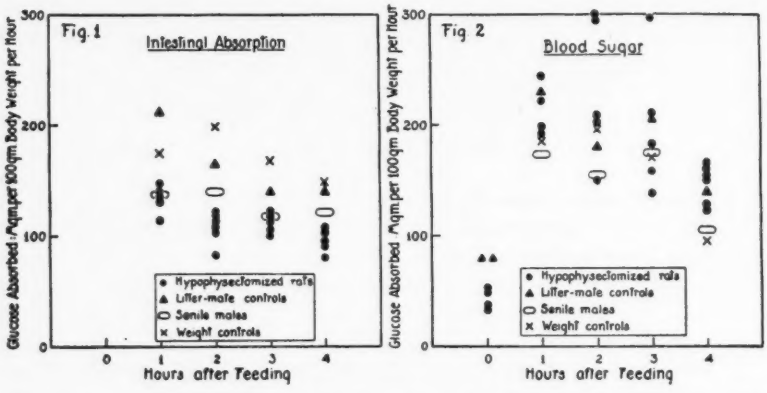
Healthy, growing (70 to 140 gm., the majority were under 100 gm.) albino male rats of a pure strain, inbred in this laboratory for 3 years were employed. The basal diet consisted of dog chow ad lib., supplemented with greens. Hypophysectomy was accomplished by the technic of Richter and Wislocki (2) with the exception that a tracheotomy, as suggested by Franseen, (3) was usually performed. Success of the operation was based upon failure of weight to increase, atrophy of testes, atrophy of thymus, atrophy of cortex of adrenal and absence of visible hypophyseal tissue at autopsy. While the completeness of hypophysectomy was not checked histologically in these experiments, similar studies have been performed elsewhere (at Cornell University Medical College) by one of us (R.A.P.) on Wistar strain rats. The chemical analyses reported in this paper were verified on the fasting animal and on the animal 3 hours after the administration of glucose. In these latter studies the success of operation was checked by serial sections of the pituitary region. Fifteen to 60 days were allowed to elapse between the operative procedure and the acute experiment. Control animals consisted of litter mates, weight mates and senile males.

The plan of experiment suggested by Cori (4) was followed. The rats were fasted 48 hours. Thereupon 0.6 gm. of dextrose per 100 gm. of body weight, in 30% solution, was fed by stomach tube and the animals were killed 1, 2, 3 and 4 hours later. The rats were fasted for 48 hours rather than for 24 hours since in mice Salter and Scharles (5) have observed a more stable fasting muscle and liver glycogen and also a cleaner gastro-intestinal tract after the longer fast. Thirty per cent glucose was used since the same authors found that a more concentrated solution would result in the development of diarrhea in a large number of the animals. Also, a less strong solution

[1] Read before the American Physiological Society, New York City, March 1934.

[2] Lucius N. Littauer Fellow in Cancer.

would necessitate over-distending the stomach with a resultant delayed elimination into the small intestine, for it is necessary to give at least 0.6 gm. per 100 gm. of body weight if one wishes to study the animals over a 4-hour period (4). The rats were killed by injecting massive doses of sodium amytal intraperitoneally as suggested by Cori (6). The latter procedure was essential since it minimized variations due to sympathetic and muscular activity



and prevented regurgitation from the stomach. Two to four minutes after injecting the sodium amytal (0.3 cc. of a 1% solution per 100 gm. of body weight) the rat was anesthetized. In the order listed, the esophagus in the neck was ligated, duplicate 0.1 cc. samples of carotid blood drawn into micropipettes, duplicate samples of liver and muscle obtained, the colon tied off just above the rectum and the bladder aspirated. Approximately two minutes were consumed in these procedures.

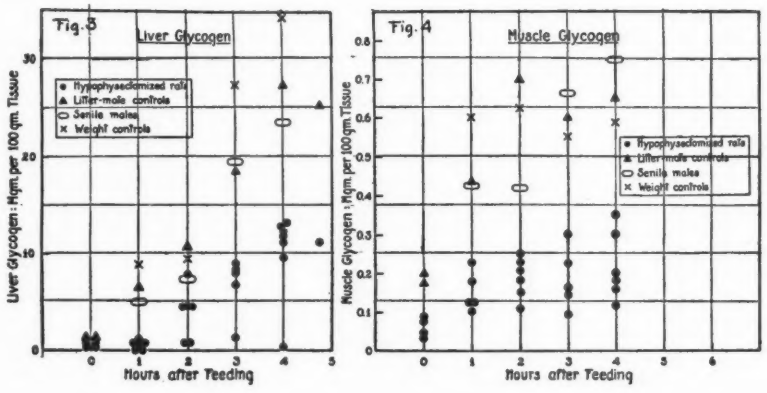
The gastro-intestinal tract was then removed en masse, washed free of blood and amytal with cold distilled water and the glucose remaining therein analyzed (4). Blood sugar values are by the Folin and Malmros micro-method, (7). Liver and muscle glycogen were determined by the Good, Kramer and Somogyi modification (8) of Pflüger's method (9) using the Shaffer-Hartmann method (10) for the glucose analysis. The 'glycogen' values reported are actually glucose. Samples of tissue weighing approximately 1.0 gm. each were obtained from the liver and muscle. Muscle glycogen was determined on the right and left quadriceps groups of muscles.

During the fasting period the rats were placed in cages having a wide-meshed floor so that all excreta would be immediately removed. During the assimilation period the rats were placed in individual cages with filter paper floors so that all excreta could be collected for sugar analysis. This was done in the manner suggested by Cori (4) for analyzing the gastro-intestinal tract contents.

The room temperature was kept between 25° and 30° C. throughout the fasting and assimilating periods since a rat deprived of its hypophysis is quite sensitive to cold. The experiments here reported were all performed in the months of May and June (19).

RESULTS

Controls. The control animals with the exception of a series of very young and a series of very old rats were litter mates of the experimental animals. The values obtained for intestinal absorption, blood sugar, muscle glycogen and liver glycogen in these experiments agree very well with those found by Cori (4) and Cori and Cori (11). Consequently, a larger series of these animals is not included. Hypophysectomy was performed on 70 to 140-gram rats. These rats failed to increase their weight or actually lost up to 15 gm. during the recovery period. Hence, it was felt advisable to include a group of 'weight controls,' 60 to 70-gram rats. Since hypophysectomy results in a preparation showing many points in similarity with senescence it was thought desirable to have a group of senile males as controls. In general, the control series of young rats absorbed more glucose and deposited it more rapidly as glycogen in their muscle and liver than did the litter-mate control group, while the reverse holds for the group of senile males. No group variations were noted in the blood sugar values.



Intestinal absorption (fig. 1). The hypophysectomized rat has a lower intestinal absorption coefficient (mg. of glucose absorbed per 100 gm. of body weight per hour) than does the normal animal. The value is about 70% of that of the litter-mate controls in this series. The explanation of this deficient absorption is not forthcoming at present. Perhaps the observation by Foster and Smith (12) that the metabolic rate (and hence the metabolic demands) are about 30% lower after hypophysectomy is significant in this regard. However, these values are of importance in showing that the hypophysectomized animal does absorb a considerable amount of fed glucose, even though it is less than that of the normal.

Blood sugar (fig. 2). While the hypophysectomized rat occasionally has a higher blood sugar than the normal after feeding glucose, still the difference does not appear to be significant unless one considers the fasting blood sugar

levels of the two series. While the controls, after fasting, were constant around 80 mg.%, the hypophysectomized animals with blood sugar values of 35 to 50 mg.% appeared to be dangerously near the hypoglycemic level. Thus the difference based on the percentage increase in blood sugar is quite striking. It was thought that the very high values obtained on the 2 and 3-hour observations might have been an error but checks on the values failed to reveal this to be so. In any event it is quite obvious that there is available glucose and this glucose appears to be disappearing from the bloodstream at about an equal rate in the two series.

Liver glycogen (fig. 3). The deposition of liver glycogen in the two groups of animals is quite different. The control values are well within the values for normal rats found by Cori (13), except for the high points on the 1, 2, 3 and 4-hour determinations which were obtained on 60 to 70-gram rats; likewise the lowest points on each of the hour determinations were obtained from a series of 'old' (270 to 350-gram) males. The difference in the controls and hypophysectomized rats is very marked. The percentage of glucose absorbed which is deposited as liver glycogen 4 hours after feeding is given by Cori (13) as 17%. Table 1 shows that hypophysectomized rats deposited only 7 to 8% of the glucose absorbed as liver glycogen.

TABLE 1. SUMMARY OF STUDIES 4 HOURS AFTER FEEDING GLUCOSE

Animal no.	Weight of rat	Glucose fed	Intestinal absorption coefficient mg. per 100 gm. per hour	Blood sugar	% of liver as glycogen	% of muscle as glycogen	Weight of liver	% of absorbed glucose deposited as liver glycogen	Total amount of glucose lost in urine and feces in 4-hour period
Hypophysectomized	gm.	mg.		mg.%			gm.		mg.
705	74.5	447	91	160	0.96	0.35	2.525	8.1	0
738	93.0	558	107	154	1.17	0.20	2.675	7.2	25.6
749	144.5	867	96	154	1.00	0.15	4.420	7.9	0.5
Control									
750	176.0	1056	143	143	2.45	0.64	4.940	12[1]	0
131	91.5	549	183	123	2.43	0.46	5.247	17.3	0

[1] Average for normal rats 17% (13). The value quoted is the lowest we have seen in a normal rat due in this case, presumably, to the small liver.

Muscle glycogen (fig. 4). Here also we have evidence of a lower glycogen deposition in the hypophysectomized animal than in the control. In addition the fasting level is much lower in the hypophysectomized series. Since the actual percentage of the body weight present as muscle is not known in the preparation studied it was felt that computation of the amount of glucose stored in the muscle as glycogen (using the ratio of muscle weight to body weight found in the normal animal) would be of dubious value. However, from the diminished activity after hypophysectomy (14) it seems likely that the mass of muscle is no greater than that of the normal rat.

Escreta. In no case was there any evidence of glucose in specimens of

the bladder urine of hypophysectomized rats (Benedict's qualitative method, 15) although the controls did at times show a trace. This absence of alimentary glycosuria in hypopituitarism was first described by Goetsch, Cushing and Jacobson (16). As was indicated in the description of methods all of the excreta was saved by placing the rats in small, individual cages with filter paper floors. Occasionally a diarrhea of mild degree would be observed and usually some urine would be voided. The largest amount of glucose recovered from the excreta of an hypophysectomized rat over a 4-hour period was 25.6 mg. This was in a 93-gram male who had absorbed 428 mg. of the 558 mg. fed, an inconsiderable error in this type of experiment.

Summary. Thus we see that the hypophysectomized rat when fed glucose, absorbs it from the gastro-intestinal tract (fig. 1) to the extent that its blood sugar increases from fasting levels more than does the control and then diminishes at about the same rate (fig. 2) yet there is a greatly diminished storage of this absorbed glucose as glycogen in the liver and the muscles (fig. 3, 4).

DISCUSSION

The fate of the absorbed glucose is not disclosed in these experiments. It is, of course, possible that glucose is stored directly as a sugar in the tissues of the hypophysectomized animal. This would not seem plausible from the work of Trimble and Carey (17), unless the blood sugar remained at a high level. A few studies of the sugar content of the skin of normal and hypophysectomized rats in these experiments 4 hours after feeding glucose failed to show more than a slight variation between the two.

The contention of Cori (6) that the glycemic level is not an adequate indicator of the degree of intestinal absorption is supported in these experiments since the hypophysectomized rat absorbs less glucose yet develops a more marked hyperglycemia than does the control (fig. 2).

Similar studies have been reported on hypophysectomized rats by Bennett (18). He found a decreased intestinal absorption of glucose and a diminished deposition of the absorbed glucose as liver and muscle glycogen.

SUMMARY

Observations on intestinal absorption, blood sugar and muscle and liver glycogen are reported on hypophysectomized albino rats; similar studies on litter mates of the above and on very young and very old rats are also given.

Intestinal absorption of glucose in the hypophysectomized rat is approximately 70% of the control animal (fig. 1), while at the same time the blood sugar values are practically identical after feeding glucose to the two preparations (fig. 2).

The deposition of absorbed glucose as glycogen in the liver (fig. 3) and in the muscle (fig. 4) of the hypophysectomized rat is much less than that in the controls.

The fasting blood sugar level of the hypophysectomized rat is much lower than that of the control.

Support is given to the contention of Cori (6) that the glycemic level is not an indication of the intestinal absorption rate.

It is a pleasure to thank the Ella Sachs Plotz Foundation for their financial aid to the Biochemical Department of the Huntington Hospital which rendered the consummation of this work possible.

REFERENCES

1. Houssay, B. A., P. Mazzocco and C. T. Rietti: *Compt. rend. Soc. de Biol.* 93: 967. 1925.
2. Richter, C. P., and G. B. Wislocki: *Am. J. Physiol.* 95: 481. 1930.
3. Franseen, C. F.: Personal Communication, 1933.
4. Cori, C. F.: *J. Biol. Chem.* 66: 691. 1925.
5. Salter, W., and F. H. Scharles: Personal Communication, 1933.
6. Cori, C. F.: *Physiol. Rev.* 11: 143. 1931.
7. Folin, O., and H. Malmros: *J. Biol. Chem.* 83: 115. 1929.
8. Good, C. A., H. Kramer and M. Somogyi: *J. Biol. Chem.* 100: 485. 1933.
9. Pflüger, E. In Abderhalden, E.: *Handbuch der biochemischen Arbeitsmethoden* 2: 1070. 1910.
10. Shaffer, P. A., and A. F. Hartmann: *J. Biol. Chem.* 45: 365. 1920.
11. Cori, C. F., and G. T. Cori: *J. Biol. Chem.* 70: 557. 1926.
12. Foster, G. L., and P. E. Smith: *J. A. M. A.* 87: 2151. 1926.
13. Cori, C. F.: *J. Biol. Chem.* 70: 577. 1926.
14. Lee, M. O., and R. G. Hoskins: Personal Communication, 1933.
15. Benedict, S. R. *J. A. M. A.* 57: 1193. 1911.
16. Goetsch, E., H. Cushing and C. Jacobsen: *Bull. Johns Hopkins Hosp.* 22: 165. 1911.
17. Trimble, H. C., and B. W. Carey, Jr.: *J. Biol. Chem.* 90: 655. 1931.
18. Bennett, L. L.: *Proc. Soc. Exper. Biol. & Med.* 34: 277. 1936.
19. Phillips, R. A., and P. D. Robb: *Am. J. Physiol.* 109: 82. 1934.

COMPARATIVE STUDIES OF GONADOTROPIC HORMONES[1]

VI. SOME EFFECTS OF LONG-CONTINUED DAILY INJECTIONS

C. F. FLUHMANN

From the Department of Obstetrics and Gynecology
Stanford University School of Medicine

SAN FRANCISCO, CALIFORNIA

THE WORK of recent years has led to the belief that gonadotropic hormones may be divided into three main groups; namely, anterior pituitary, chorionic, and equine. This is a further report (1, 2) of certain changes in female rats following the daily administration of such substances over periods ranging from 28 days to one year, and which attest to the probable correctness of this classification.

PROCEDURE

Three preparations were employed in these experiments. The first was made from desiccated sheep anterior pituitary tissue by extracting with a sodium acetate-acetic acid buffer, pH 4.2 to 4.5, and reprecipitating with ethyl alcohol, according to the method originally described by Wallen-Lawrence and Van Dyke (3). The second, chorionic hormone, was prepared in a similar manner from pooled specimens of blood obtained from women in various stages of pregnancy (4). The third was a diluted preparation of pregnant mares' serum, known as gonadin (Cutter Laboratories), and made essentially in the manner developed by Cartland and Nelson (5). Judging by its effect on ovarian weight in 5-day experiments, each extract was comparatively weak and produced ovaries of only 0.019 to 0.036 gm. In all instances, injections were begun when the rats were 21 to 23 days of age and the daily dosage was 0.25 cc.

At autopsy, attention was directed chiefly to the weight and histologic modification of the ovaries and uteri and a record of the weight of the hypophyses, thyroids and adrenals was also kept. The hypophyses were fixed in Zenker's solution and stained with eosin-hematoxylin, but cell counts and studies with special staining methods were not conducted. The breast changes will be reported in detail by Dr. N. J. Howard in a separate communication.

Sheep Anterior Pituitary Extract

A series of 19 rats was given the anterior pituitary extract for periods varying up to 179 days, and the effect on organ weights is given in table 1.

[1] This study was supported in part by the Rockefeller Fluid Research Fund of Stanford University School of Medicine, and by a grant from the Committee for Research in Problems of Sex, National Research Council. My thanks are due to Mr. John J. Kan and Mr. Pierre Lassègues for their technical assistance, to Mr. D. A. Wonder and the Cutter Laboratories for the gonadin used in this study, and to Eli Lilly & Co. for a supply of desiccated sheep anterior pituitary glands.

The two most remarkable changes were concerned with the gonads and the anterior hypophysis. At 63 and 94 days the ovaries weighed .114 and .107 gm. respectively, but there subsequently occurred a marked regression, so that after 150 days the average was only .031 gm. and after 179 days it was .042 gm. At the same time, there was a gradual increase in the gross weight of the hypophysis.

TABLE 1. ORGAN WEIGHTS OF RATS GIVEN DAILY INJECTIONS OF A SHEEP ANTERIOR PITUITARY EXTRACT

Days injected	Age at autopsy	No. of rats	Body weight	Thyroid	Adrenals	Uterus	Ovaries	Hypophysis
	days		*gm.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
5	26	26	37			49	36	
63	84	6	135				114	
94	115	3	174	31	77	206	107	13
150	171	5	190	31	52	139	31	14
179	200	5	206	31	64	274	42	16

The histologic changes were in keeping with the modifications in the weights of ovaries and pituitary glands and corroborated findings previously reported by Collip et al. (6, 7), Thomson et al. (8) and Selye et al. (9). In the

Fig. 1. OVARY OF RAT INJECTED DAILY FOR 94 DAYS WITH AN ANTERIOR PITUITARY (SHEEP) GONADOTROPIC HORMONE. Fig. 2. OVARY OF RAT INJECTED DAILY FOR 150 DAYS WITH AN ANTERIOR PITUITARY (SHEEP) GONADOTROPIC HORMONE. (Same magnification as fig. 1.) Fig. 3. OVARY OF RAT INJECTED DAILY FOR 150 DAYS WITH AN ANTERIOR PITUITARY (SHEEP) GONADOTROPIC HORMONE. (Higher magnification than fig. 1, 2.) Fig. 4. OVARY OF RAT INJECTED DAILY FOR 365 DAYS WITH AN EXTRACT OF CHORIONIC GONADOTROPIC HORMONE. (Same magnification as fig. 1, 2.) Fig. 5. METAPLASTIC CHANGES IN THE UTERUS OF A RAT INJECTED DAILY FOR 272 DAYS WITH AN EXTRACT OF CHORIONIC GONADOTROPIC HORMONE. Fig. 6. METAPLASTIC CHANGES IN THE UTERUS OF A RAT INJECTED DAILY FOR 365 DAYS WITH AN EXTRACT OF CHORIONIC GONADOTROPIC HORMONE. Fig. 7. OVARY OF A RAT INJECTED DAILY WITH AN EXTRACT OF EQUINE GONADOTROPIC HORMONE (GONADIN) FOR 123 DAYS. (Same magnification as fig. 1, 2, 4.)

group autopsied in 94 days, the ovaries were enlarged and contained many small corpora lutea, a few developing graafian follicles, lutein cysts, atretic follicles and cysts lined with fibrous tissue, while the stromal cells were

hypertrophied (fig. 1). The anterior lobes of 2 rats clearly showed an increase of basophiles, while 'castration cells' were present in the third. After 150 and 179 days the ovaries showed a marked atrophy. This was apparent in the stroma, which contained many 'deficiency cells' (wheel cells) such as occur after hypophysectomy (10), and in some instances there was an extensive hyaline degeneration (fig. 2, 3). In most cases, no corpora lutea nor normal graafian follicles were present, but a few atretic follicles were visible. In keeping with the ovarian atrophy, the anterior hypophysis contained numerous vacuolated basophiles or 'castration cells.'

Chorionic Gonadotropic Hormone

The preparation made from blood of pregnant women was administered to a group of 38 rats for periods ranging from 28 to 365 days.[2] From table 2 it is seen that this resulted in a progressive increase in the average weights of the ovaries until the 272nd day. The ovaries of the 2 animals injected for

TABLE 2. ORGAN WEIGHTS OF RATS GIVEN DAILY INJECTIONS OF CHORIONIC GONADOTROPIC HORMONE

Days injected	Age at autopsy	No. of rats	Body weight	Thyroid	Adrenals	Uterus	Ovaries	Hypophysis
	days		*gm.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
5	26	20	34			98	28	
28	49	11	75	14	24	221	71	4
59	80	7	103	18	38	313	137	9
90	111	9	152	28	45	492	240	12
5[1]	26	7	43			97	38	
193	214	5	188	36	67	861	232	13
272	293	4	241	50	86	756	469	25
365	386	1	190[2]	46	68	832	205	8
365	386	1	253	47	59	1232[3]	236	34

[1] Increase of daily dosage after 175th day.
[2] This animal had lost weight and been ill for about one month before autopsy.
[3] Pyometra.

365 days had regressed, but still weighed approximately 4 times more than those of normal adult animals. The hypophyses of the rats killed in 272 days, and one of those in 365 days, also showed a marked increase in weight.

The histologic examination, both of ovaries and anterior hypophysis, presented very distinct differences from the rats receiving the anterior lobe extract. The structures in the ovaries corresponded with those previously noted in shorter experiments and consisted of developing follicles, cysts lined with lutein cells, large corpora lutea, and a hypertrophy of the stromal cells (11, 12). These changes are so characteristic that they may be used as criteria for the differentiation of the chorionic from other gonadotropic hormones. However, when the injections were continued for 193 days and longer, a new structure appeared consisting of cysts lined solely with fibrous tissue (fig. 4). As the experiment continued they increased both in size and

[2] The concentration of the extracts was increased during the latter part of the experiment. During the first 175 days a dilution was used which in 5 days induced an average ovarian weight of .028 gm. but subsequently it was employed at a strength which produced ovaries of .038 gm.

number. For instance the ovaries of one rat injected for 272 days weighed 1.137 gm., each gonad consisting almost entirely of such cysts. After 365 days the ovaries contained a few corpora lutea, many fibrous cysts and only an occasional developing follicle. The stroma, in addition, presented an unusual appearance in that it was deeply infiltrated with round cells and macrophages containing old blood pigment. Since these ovaries also had decreased in weight, such changes possibly may point to a progressive disintegration and had the experiment continued for a longer period an extensive atrophy might have resulted. The enlarged anterior lobes of the rats injected for 272 and 365 days showed no increase of basophilic cells, but apparently corresponded with those found by Severinghaus (13), Wolfe et al. (14), and Wolfe (15) following the injection of urine from pregnant women, namely, an increase of chromophobes and a decrease in eosinophiles. However, careful cell counts were not conducted and special staining methods were not employed.

The weights of the uteri followed fairly closely the weights of the ovaries but two changes, reported by other investigators as resulting from the prolonged administration of estrogenic substances, must be mentioned. First, the uteri of 2 rats injected for 193 days and one for 365 days showed pyometra with an extensive intra-abdominal inflammatory reaction. Secondly, in two instances, one a rat injected for 272 days and another for 365 days, there were extensive metaplastic changes in the uterine mucosa, which was transformed into a type of squamous epithelium (fig. 5, 6).

Equine Gonadotropic Hormone

The equine gonadotropic hormone was given to 13 rats for periods varying from 28 to 123 days. As indicated in table 3, the ovaries enlarged slightly but there was no marked progressive increase in weight such as was observed with the other two extracts. The uteri, however, showed very early a pronounced weight increase out of all proportion to that of the ovaries. The

TABLE 3. ORGAN WEIGHTS OF RATS GIVEN DAILY INJECTIONS OF A GONADOTROPIC EXTRACT OF PREGNANT MARES' SERUM

Days injected	Age at autopsy	No. of rats	Body weight	Thyroid	Adrenals	Uterus	Ovaries	Hypophysis
	days		*gm.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
5	26	6	29			70	19	
28	49	3	79	20	41	461	32	4
60	81	3	114	22	59	449	42	8
90	111	3	128	31	70	459	45	10
123	144	4	136	27	54	508	69	10

hypophysis remained within normal limits, which appears to be contrary to the findings of Evans et al. (16), who observed a 3-fold increase in the weight of the hypophysis after 30 days' treatment. However, the two experiments are not comparable since these authors employed the equine hormone at a much greater concentration. In 30 days they obtained ovaries averaging as much as 1.579 gm. in weight.

The histologic examination of the hypophysis and uterus failed to show any striking deviation from the normal. The ovaries resembled the normal organ of adult female rats, containing developing follicles and corpora lutea, and the stromal cells were hypertrophied (fig. 7).

DISCUSSION

It is necessary, in considering the results reported in this study, to remember the fundamental importance of the daily dosages employed in long-continued experiments. Collip et al. (9) directed attention to the fact that with both anterior pituitary and chorionic hormones there are two different stages in the effects on the gonads and the hypophysis. First, there is a stage during which the ovaries are enlarged, and second, a period when regression occurs. In addition, there is good reason to believe that the higher the daily dosage the sooner will the first stage terminate and the second appear.

In the present study, the strength of each extract was slight insofar as immediate effects on ovarian weight are concerned and for this reason it is felt that the findings are open to direct comparison. When this is done, it is found that fundamental differences exist in the biologic characteristics of the three groups of hormones.

a), With the sheep anterior pituitary extract the ovaries reached a maximal weight far in excess of the normal in about 100 days and subsequently became markedly atrophic. The anterior lobes of these animals gradually increased in size as the ovarian atrophy proceeded and eventually assumed the histologic appearance seen after castration. The uteri increased in weight but never to the same extent (in proportion to the increase of ovarian weight) as with the other two gonadotropic factors investigated.

b), The chorionic gonadotropic hormone produced a progressive increase in ovarian weight which lasted longer and was much greater than with the anterior lobe extract. Eventually, it regressed but it still remained, even after one year's treatment, almost four times greater than normal controls. Histologically, the ovaries did not show the complete atrophy seen in the first series and they contained large cysts lined with fibrous tissue and corpora lutea. The anterior hypophysis enlarged but the histologic picture was not of the post-castration type seen with the hypophysial extract. The uteri also showed greater increases in weight in proportion to the size of the ovaries.

c), Injection of the equine gonadotropic hormone at the dosage employed produced singularly negative results. Although it is the most potent gonadotropic hormone available, its daily injection for 123 days yielded ovaries weighing scarcely more than those of control rats of the same age. The anterior lobes of these animals showed no deviation from the normal. However, in proportion to the size of the ovaries, the uteri enlarged more than with either of the other two preparations.

SUMMARY

The long-continued daily injection of various gonadotropic substances to rats 21 to 23 days of age at the start of the experiment indicates funda-

mental differences which justify their classification into the three groups of, anterior pituitary, chorionic and equine gonadotropic hormones.

REFERENCES

1. Fluhmann, C. F.: *Proc. Soc. Exper. Biol. & Med.* 34: 691. 1936.
2. Howard, N. J.: *Proc. Soc. Exper. Biol. & Med.* 34: 732. 1936.
3. Wallen-Lawrence, Z., and H. B. Van Dyke: *J. Pharmacol. & Exper. Therap.* 43: 93. 1931.
4. Fluhmann, C. F.: *Proc. Soc. Exper. Biol. & Med.* 29: 1193. 1932.
5. Cortland, G. F., and J. W. Nelson: *Am. J. Physiol.* 122: 210. 1938.
6. Collip, J. B., H. Selye and D. L. Thomson: *Proc. Soc. Exper. Biol. & Med.* 31: 682. 1934.
7. Collip, J. B., H. Selye and J. E. Williamson: *Endocrinology* 23: 279. 1938.
8. Thomson, D. L., H. Selye and J. B. Collip: *Am. J. Physiol.* 109: 105. 1934.
9. Selye, H., J. B. Collip and D. L. Thomson: *Proc. Soc. Exper. Biol. & Med.* 31: 487. 1934.
10. Selye, H., J. B. Collip and D. L. Thomson: *Endocrinology* 17: 494. 1933.
11. Fluhmann, C. F.: *Endocrinology* 17: 550. 1933.
12. Fluhmann, C. F.: *Am. J. Physiol.* 106: 238. 1933.
13. Severinghaus, A. E.: *Anat. Rec.* 60: 43. 1934.
14. Wolfe, J. M., E. T. Ellison and L. Rosenfeld: *Anat. Rec.* 60: 357. 1934.
15. Wolfe, J. M.: *Anat. Rec.* 63: 1. 1935.
16. Evans, H. M., M. E. Simpson and M. McQueen-Williams: *Univ. Calif. Pub. in Anat.* 1: 61. 1934.

OBSERVATIONS ON NITROGEN AND CALCIUM BALANCES AS AFFECTED BY GROWTH AND GONADOTROPIC HORMONES ADMINISTERED FOR SHORT PERIODS TO GROWING CHILDREN

J. A. JOHNSTON AND JOHN WALKER MARONEY
From The Henry Ford Hospital
DETROIT, MICHIGAN

RETENTION of nitrogen and of minerals is accepted as a metabolic characteristic of growth. Under normal circumstances, there is a definite limit to the quantity of each that can be retained, and increasing retentions will parallel increasing intakes only within definitely fixed limits, so that experimentally there can be readily produced the paradox of diminished retention following increasing intake when this limit has been reached (1). A preliminary period of withdrawal of these items results in an increase in retention.

A second factor conditioning retention in man is age. Not only does the ability to retain cease with the completion of growth, but within the growth span there are marked quantitative variations in retention. Notably high storage marks the periods of early infancy and pre-puberty, and an abrupt falling off in retention is evident almost synchronously with the menarche.

In studies on the effect of the essential amino acids, one conditioning factor has been noted in their administration to animals previously deprived of them as contrasted with those already receiving normal amounts; striking growth effects obtained in the former while none was evident in the latter.

In the field of hormones, it is well accepted that an effect will be conditioned by the presence or absence of a defect. This point is stressed for thyroid by Janney (2) and, in the case of the growth hormone, hypophysectomized animals show a more striking response than do normals.

The literature on nitrogen and calcium balances as influenced by growth hormone brings out the following points. *a*) The retention of nitrogen induced by the hormone is to a large extent only transiently held, though some of it does seem permanent (3). *b*) Gaebler's largest retentions in the adult dog were obtained when the control balance was small or even negative (4), suggesting that the limiting factor noted in the dietary experiments may hold here likewise. *c*) Attempts at duplicating the results quantitatively in the same animal have usually met with failure, though after a 2-month interval Gaebler could again repeat his initial finding. This might be interpreted as similar to the failure to add to stores already saturated, or an antihormone effect (5) may have been induced. *d*) Greater effects resulted from the administration of the hormone to animals deprived of their own pituitaries than to normals (6). Regarding calcium, Pugsley and Andersen (7) were able to convert a negative calcium balance on a low diet following hypophysectomy into a positive one by administering

growth hormone, but Teel and Cushing (8) report an increased urinary excretion of calcium with diminished phosphorus output when the hormone was administered to the normal animal. Regarding the effect on nitrogen of the APL substance, Gaebler (9) reported "changes simulating the action of growth preparations . . . nitrogen storage with subsequent loss . . . in three experiments, but these were delayed and less marked than those which follow administration of growth hormone preparations."

The observations here reported were made on a group of children hospitalized for a variety of conditions, some of which may have involved a defect in their own pituitary secretions; others showed no glandular disturbance, and some were normal. Ages were from 10 months to 19 years. The pre- and post-menarcheal periods are represented. Control periods show instances of both negative and positive balances.

METHODS

In the tables 'Standard diets 1–6' refer to diets prepared from staple foods, changing each day for 6 days in rotation. The proportions of each in percentage calories were 15% protein, 35% fat and 50% carbohydrate. At the end of each 6-day period, diets were analyzed for calcium and nitrogen using one-sixth of the content of each of the 6 diets in the final sample. In some of the earlier experiments in 1935 and 1936, where no specific reference is made to 'Staple diets 1–6,' mixed foods were used and 50 analyses made to determine the average nitrogen content as described in another communication (10). A few cases received a simpler diet whose mineral content was known and which was not varied daily. The chief source of calcium was milk in the form of Klim. In preliminary periods an attempt was made to give a number of calories on which the child was satisfied and gained in weight. The growth preparation used was antuitrin-G and the anterior pituitary-like substance antuitrin-S, both kindly supplied by Parke, Davis & Co. Each cubic centimeter of the growth substance contained 10 R.U., and of the sex hormone 500 R.U. It should be pointed out[1] that the doses used may have been inadequate to produce unequivocal results and that the antihormone effect might be related to the amount of inactive protein in the material used.

Determinations of basal metabolism were made by the gasometer method. The accuracy of analysis of the expired air was checked by frequent analyses of the outside air. The results were calculated from the oxygen consumption and the total respiratory quotient. The standards used were those of Boothby and Sandiford, but there are also recorded the basal calories for 24 hours.

CASE REPORTS

Genevieve B. Age was 8 years, height 107 cm., weight 37 lb. This child was an ateleotic dwarf. The wrist plates showed normal epiphyses. The first B.M.R. was +6. See table 1, figure 1.

The first course of antuitrin-G treatment consisted of 10 injections on alternate days of 5 cc. of the hormone. A definite increase in retention was evident following

[1] A suggestion of Dr. M. O. Lee.

TABLE 1

Genevieve B. Born 3/10-26 — Diet 1400 Calories 15-35-50% — 202047

Dates 1934	NITROGEN			B.M.R.	Wt.	Ht.	Remarks
	Output	Intake	Balance				
8/13-15	9.26	9.38	+0.12	+6	37	107 cm.	
8/16-18	8.58	9.38	+0.80				
8/19-22	7.07	9.38	+2.31		37		
			+1.20	Mean Balance			
8/23-25	5.63	9.38	+3.75				Ant.-G 5 cc. 8/23-25-27-29 & 31
9/1-3	5.66	9.38	+3.67	−2			Ant.-G. 2½ cc. 9/2
9/4-6	7.24	9.38	+2.14				
9/7-9	8.84	9.38	+0.54				Ant.-G. 5 cc. 9/7
9/10-12	8.43	9.38	+0.95				Ant.-G. 5 cc. 9/11
9/13-17	9.94	9.38	−0.77				Ant.-G. 5 cc. 9/13 & 15
			+1.59	Mean Balance			
9/18-19	10.25	9.38	−0.87				
9/20-22	9.81	9.38	−0.43				
9/24-25	8.74	9.38	+0.64		38		
9/26-28	9.17	9.38	+0.21				
			−0.11	Mean Balance			
10/2-4	10.92	9.38	−1.54				Ant.-G. 5 cc. q. other day
10/5-7	8.98	9.38	+0.40				10/3-15
10/8-10	8.98	9.38	+0.40				
10/11-13	9.22	9.38	+0.16	−4	39½	108 cm.	
10/14-16	9.70	9.38	−0.22				
10/17-19	9.40	9.38	−0.02				Ant.-G. 5 cc. 10/18 & 20
10/20-22	9.07	9.38	+0.31		39¾		
			−0.07	Mean Balance			
10/23-25	8.89	9.38	+0.49				
10/26-28	7.62	9.38	+1.76				
10/29-31	9.79	9.38	−0.41		40		
			−0.60	Mean Balance			

the first 6 injections followed by a fall in retention during the remainder of the month and coincident with 4 additional injections. In the second control period a negative balance was maintained suggesting a complete loss of retained nitrogen. A second course of the hormone was accompanied by a negative balance. This might be interpreted as a continuance of the 'unloading' phenomenon, rather than a definite hormone effect, though in any case there is an absence of any anabolic effect. A final third control period showed a slightly positive balance.

There were shown in this experiment, (*a*), the loss of much of the transiently held nitrogen, and (*b*), the loss of effect on repetition of the injections, suggesting the development of antihormone. A similar effect was noted with antuitrin-G in the case of *Geraldine H.* and *Stanley R.*

Stanley R. Age was 13 years, height 56 in., weight 74 lb. Bone age was normal. During a study published elsewhere (11) it was recorded that the removal of a focal infection was followed by a marked improvement in nitrogen retention and a fall

in basal metabolism, this particular type of infection being studied as an example of one without fever. See table 2, figure 2.

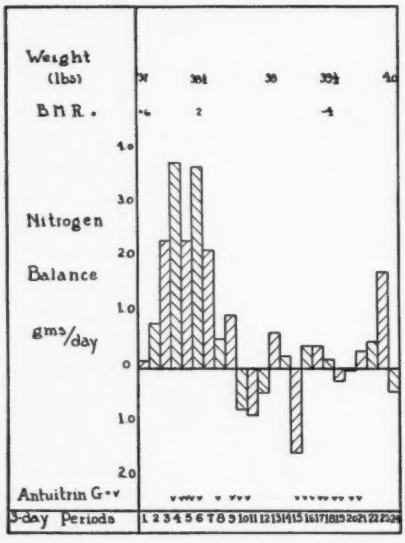


Fig. 1

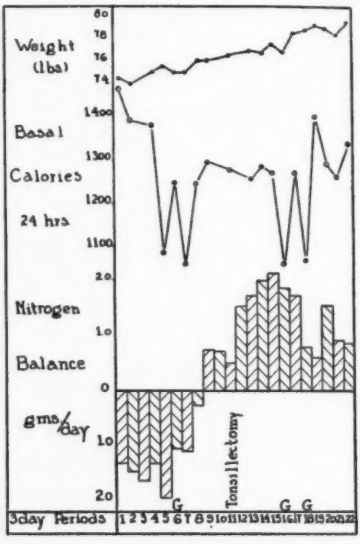


Fig. 2

Fig. 1. *Genevieve B.* Ateleotic Dwarf. Each bar represents a 3-day balance, and the balances are continuous. Each arrow indicates an injection of 5 cc. of antuitrin-G. There are indicated the loss of much of the increased retention, and the failure of effect of the second series. Fig. 2. *Stanley R.* Continuous nitrogen balance. Each bar represents 3 days. "G" in the 6th period refers to the injection of 3 cc. daily for 3 days of antuitrin-G. "G" in the 16th period denotes the injection daily for 3 days of 5 cc., and in the 18th period to the injection daily for 3 days of 10 cc.

The entire observation on this boy covered 69 days. Antuitrin-G was administered before and after tonsillectomy. The first administration seemed to convert a negative balance into a positive one. Three injections of 3 cc. each were given daily in the 6th period; improved retentions are noted in the 8th, 9th, 10th and 11th periods. The tonsillectomy was then performed and another control period run. With the balance still strongly positive, antuitrin-G in the 16th period had no immediate effect; repeated again in the 18th period there was a slightly diminished retention.

The question of interpretation involves the consideration of whether the hormone effect may be conditioned by the state of the nitrogen stores. The question likewise arises, as it does in other cases in the series, of a failure to secure any effect on repetition of the injections, and whether this is evidence for the development of antihormone or again may be accounted for by the fact that the 2nd and 3rd series of injections were given when the balances were positive.

Helen S. Age was 15 years, 10 months, height 61 in., weight 141½ lb. This child presented a typical example of the Lawrence-Moon-Biedl syndrome—obesity, retinitis pigmentosa, mental retardation and deformity of the toes. During the course

TABLE 2

Stanley R. 257679 Born 3-6-24	Standard Experimental Diet 1-6 2200 Cal. 15-35-50%									Ht. 56 in. Wt. 74½ lb. Est. Wt. 78 lb.
1937 Dates	NITROGEN					Cal/sq. m/hr.	Basal Cal.	Wt.	Ht.	Remarks
	Output	Intake	Balance	B.M.R.	R.Q.					
7/4-6	14.59	13.27	−1.32	+13	.96	52.3	1469	74½	56	
7/7-9	14.64	13.17	−1.47	+ 8	.78	49.0	1390	74	56	
7/10-12	14.92	13.27	−1.65							
7/13-15	14.54	13.20	−1.34	+ 6	.74	49.1	1378	75	56	
7/16-18	15.20	13.27	−1.93	−16	.80	38.6	1082	75½	56	
			−1.54	Mean Balance						Control Period
7/19-21	14.35	13.27	−1.05	− 5	.83	44.1	1248	75	56	Ant.-G. 3 cc. qd.
7/22-24	14.27	13.17	−1.10	−18	.83	37.5	1062	75	56	
7/25-27	13.53	13.27	−0.26	− 4	.82	44.1	1246	76	56	
7/28-30	12.54	13.27	+0.73	− 1	.78	45.7	1296	76	56	
7/31-8/2	12.56	13.27	+0.71							
8/3-5	11.56	12.03	+0.47	− 3	.77	44.7	1277	76½	56	Tonsillectomy 8/5
			+0.11	Mean Balance						
8/21-23	11.74	13.27	+1.53							
8/24-26	11.53	13.27	+1.74	− 3	.81	44.5	1260	77	56½	
8/27-29	11.28	13.27	+1.99	− 2	.76	45.3	1282	76¾	56½	
8/30-9/1	11.15	13.27	+2.12	− 5	.78	44.1	1270	77½	56½	
			+1.29	Mean Balance						
9/2-4	11.43	13.27	+1.84	−10	.82	41.3	1169	76¾	56½	Ant.-G. 5 cc. qd.
9/5-9	11.55	13.27	+1.72	− 4	.80	44.2	1272	78½	56½	
9/10-12	12.48	13.27	+0.79	− 5	.80	44.0	1077	78¾	56½	Ant.-G. 10 cc. qd.
9/13-16	12.67	13.27	+0.60	+ 1	.80	46.5	1399	79¼	56¾	
9/17-19	11.76	13.27	+1.51	− 3	.80	44.8	1290	79	56¾	
9/20-22	12.43	13.27	+0.84	− 5	.81	43.7	1258	78½	56¾	
9/23-25	12.39	13.27	+0.88	± 0	.81	46.1	1338	79½	56¾	
			+0.95	Mean Balance						

of observation, a typical vertebral epiphysitis (Scheuermann's disease) with marked kyphosis developed. The results of Wassermann and tuberculin tests were negative. Roentgenograms at the age of 15 years, 10 months, when the first observation was made and at 17 years, 9 months, when the second observation was made, showed closed epiphyses of the wrists but other epiphyses were not studied. See table 3, figure 3.

The first observation, during which 5 cc. of antuitrin-G was administered daily for 4 days showed an increased retention of nitrogen and calcium, which was not sustained in the after period. The period was too short for the calcium figures to be significant but they are included because the effect was so extreme. The second observation 2 years later showed a rise in the nitrogen retention and a very marked decrease in calcium retention.

In the first experiment the control period for both elements showed a negative balance; in the second, the control showed a positive calcium and a negative nitrogen balance.

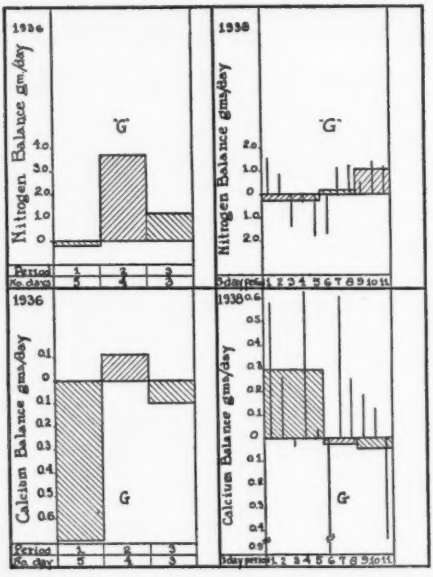


Fig. 3. *Helen S.* Lawrence-Moon-Biedl Syndrome. In the 1936 observations, the cross-hatched blocks denote an average of the entire periods indicated. In the 1938 observations, single bars represent 3-day balances, and the cross-hatched blocks the average of all periods contained within them. In the 1936 study, she received 5 cc. of antuitrin-G daily for 4 days. In the 1938, she received 10 cc. on the second day of the 6th and 8th periods.

TABLE 3

Helen S. 169778 Born 4-17-20 Weight 141½ lb. Gamble Diet 1485 Cal.

1936 Dates	NITROGEN Output	Intake	Balance	CALCIUM Output	Intake	Balance	B.M.R.	Basal Cal.	Wt.	Ht.	Remarks
1–25							−14	1322	146½	61	
2–7 to 11	7.84	7.69	−0.15	1.514	0.825	−0.689					
2–12 to 15	4.03	7.76	+3.73	0.716	0.830	+0.114					Ant.-G. 5 cc. qd.
2–16 to 18	6.63	7.78	+1.15	0.933	0.836	−0.097					
1938											
1–22 to 23	10.33	11.98	+1.65	0.613	1.199	+0.586	−10	1366	157	61	
1–24 to 26	11.31	12.17	+0.86	0.936	1.202	+0.266	−12	1311	156½	61	
1–27 to 29	13.78	12.36	−1.42	1.237	1.205	−0.032	−12	1317	156½	61	
1–30 to 2–1	12.72	12.36	−0.36	0.570	1.205	+0.635	−10	1337	155	61	
2–2 to 4	14.12	12.36	−1.76	1.170	1.205	+0.035	−10	1348	154½	61	
	Mean Balance		−0.21			+0.298					
2–5 to 7	14.07	12.36	−1.71	2.147	1.205	−0.942	−13	1293	154½	61	Ant.-G. 10 cc. 2–6 to
2–8 to 10	11.25	12.36	+1.11	0.590	1.205	+0.615	−13	1308	155	61	
2–11 to 13	11.14	12.36	+1.22	0.953	1.205	+0.252	− 8	1369	155½	61	Ant.-G. 10 cc.
	Mean Balance		+0.21			−0.025					
2–14 to 16	11.80	12.36	+0.56	1.016	1.205	+0.189	−15	1272	154	61	
2–17 to 19	10.94	12.36	+1.42	1.080	1.205	+0.125	−13	1298	154	61	
2–20 to 22	11.25	12.42	+1.17	1.625	1.187	−0.438	−13	1279	151½	61	
	Mean Balance		+1.09			−0.041					

There is a suggestion here as elsewhere in the series that positive effects were obtained when a negative balance existed before treatment. It must also be taken into account, however, that a conditioning factor may have been

age and that, while both studies were post menarchial and were made when wrist plates showed closed epiphyses, growth had probably not ceased when the first study was made.

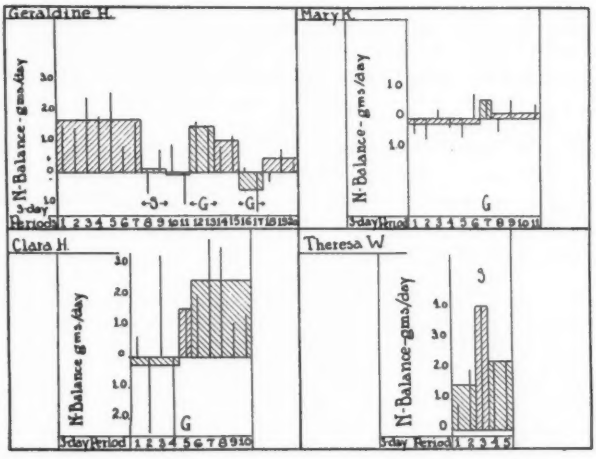


Fig. 4. Charts of 4 girls whose clinical history suggested a hormone defect. Each bar represents a 3-day balance; a cross hatched block is the average of all bars contained within it.

TABLE 4

Dates	No. of days	NITROGEN Output	Intake	Bal.	B.M.R.	R.Q.	Total cal.	Weight	Antuitrin
Theresa W.									
1935									
1/26–1/31	6	9.19	10.60	+1.41	− 6			99	
2/1–4	4	6.56	10.60	+4.04	− 9				Ant.-S. 10 cc.2/1–2–3
2/5–10	6	8.55	10.60	+2.05					
Clara H.									
1937									
7/8–7/19	12	13.44	13.18	−0.26	− 9	.87	1138	81	
7/20–22	3	11.70	13.27	+1.57	−12	.82	1073	82	Ant.-G. 3 cc. qd.
7/23–8/5	15	10.86	13.27	+2.41	− 7	.88	1141	85	
Mary K.									
1/27–29	18	13.39	13.20	−0.19	− 1	.80	1527	112½	
2/14–16	3	12.60	13.20	+0.60	− 3	.83	1553	115	Ant.-G. 3 cc. qd.
2/17–3/1	13	13.03	13.20	+0.17	− 8	.78	1440	113½	
Geraldine H.									
1937									
4/11–5/3	23	9.01	10.75	+1.74	− 6	.81	1063	58½	
5/4–9	6	10.22	10.31	+0.09	− 1				Ant.-S. 3 cc. qd.
5/10–15	6	10.79	10.76	−0.03	−14	.79	967	60½	
5/16–21	6	9.26	10.80	+1.54	− 1	.77	1130	60	Ant.-G. 3 cc. qd.
5/22–27	6	9.75	10.80	+1.05	−10	.80	1018	61	
5/28–6/2	6	11.32	10.80	−0.52	+ 1	.77	1145	61½	Ant.-G. 3 cc. qd.
6/3–11	9	10.64	10.94	+0.30	− 3	.85	1120	62	

Geraldine H., Mary K., Clara H., and Theresa W. There are grouped together in table 4 and figure 4 the observations on 4 girls whose histories suggested the possibility of a hormone defect. In one, Dr. J. P. Pratt considered the uterus to be infantile. In two of these an anabolic effect on nitrogen followed the use of antuitrin-G; in one a similar response occurred to antuitrin-S, and in the fourth, a katabolic effect followed antuitrin-S, an anabolic effect the first course of antuitrin-G, but with the second course, the balance became negative. This child had not begun to menstruate nor had she shown the development of any of the secondary sex characteristics.

The interpretation of these suggests the positive effect conditioned by a defect. The depression of retention in the fourth case with antuitrin-S was obtained before the menarche at a time when the growth impulse is at its peak. We have found at puberty a sharp reduction in both nitrogen and calcium retention following the onset of menses.

Geraldine H. Age was 12 years, height 55¾ in., weight 56½ lb. This patient was seen because of anorexia and weight loss. She showed no evidence of secondary sex characteristics. No infection was found. The first B.M.R. of −15 would have to be interpreted with extreme malnutrition in mind.

A diminished retention seemed to follow the administration of antuitrin-S. Storage followed the first injections of antuitrin-G though these were not greater than the initial controls. The second series of antuitrin-G was followed by diminished retention.

Mary K. Age was 13 years, height 62½ in., weight 112 lb. This child was seen at 12 years, 3 months. Menstrual periods had been regular for 6 months, following which they had ceased for 7 months. During that time she had gained 9 lb. Gynecological observations revealed normal uterus and ovaries.

She received 3 cc. of antuitrin-G daily for 3 days. The balances throughout the entire experiment showed a range between −0.71 and +0.87 and the apparent slight increase during the administration is of doubtful significance. Although a single balance just before administration was higher than that obtained during the administration of the hormone, this last was higher than 5 out of 6 controls and the after period average was higher than the control period average. She began to menstruate 3 days before the injection and ceased the day of the first injection, but resumed the second day. The injections provoked nausea and were discontinued. The highest balance in the series was noted when the menstrual period was in progress, just before the injection. On thyroid she now has normal menses.

Clara H. Age was 14 years, height 58¼ in., weight 76 lb. In 1936, this child was seen because of malnutrition. She showed chronic tonsillitis and a healed childhood type of tuberculosis. The B.M.R. was −15 but rose to +2 without thyroid on a high caloric diet. Glucose tolerance and phosphorus curves were normal and the bone age was between 16 and 18 years. In 1937, when the present studies were made she gave a history that menses had started 4 months previously, the period lasting 3 days; there had been a second period 3 months later, also lasting 3 days and accompanied by low back pain. The nitrogen balances with antuitrin-G showed a definite increase unaccompanied by any change in the B.M.R. The effect here may be conditioned by the fact that the balances in the control period were negative, or by the possibility that the subject had a pituitary defect; the latter might be responsible for the former.

Theresa W. Age was 14 years, height 62½ in. weight 99 lb. This child's menarche

had occurred in May, 1934. She menstruated in July and September and then ceased to menstruate. Examination by a gynecologist showed an underdeveloped uterus and cervix. The B.M.R. was −6. No resumption of menses followed the injections of antuitrin-S nor was any effect noted subsequently from thyroid until August, 1935. A definite increase in the retention of nitrogen followed the administration of antuitrin-S, and in the after period this was not entirely lost.

Lucille R., *Dolores E.*, and *Russell J.* There are grouped together in table 5, figure 5, three conditions characterized by disturbances of growth in which we felt no definite permanent anabolic effect followed the use of antuitrin-G. In *Lucille R.*, an ateleotic dwarf aged 19 years, there was an actual diminution of nitrogen retention, which might be related to her age, 19 years. In the cretin getting no thyroid, a delayed

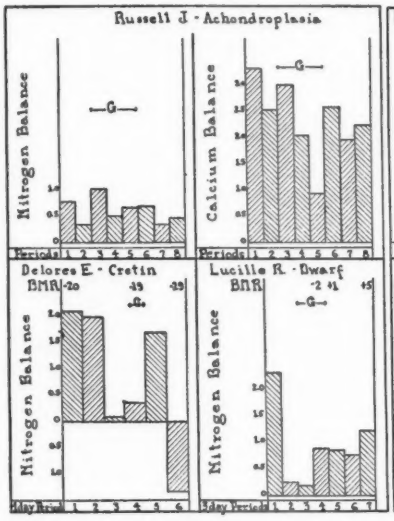


Fig. 5

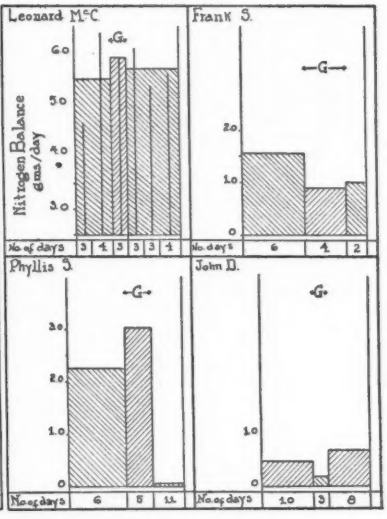


Fig. 6

Fig. 5. Chart of the balances of 3 subjects with growth disturbances. In the achondroplastic, the balances are not continuous, but because of the simplicity of this particular diet (Klim alone) probably have more significance than would otherwise be the case with 'spot' balances. Each block is a period and the duration is indicated in table 5. The achondroplastic received 1 cc. of antuitrin-G every other day. The cretin received 3 cc. daily for 3 days. *Lucille R.* received 5 cc. daily for 3 days. Fig. 6. Nitrogen balances on 4 children whose control balances were positive, and so far as we knew, had no pituitary defect. This is open to question in the Perthes's case.

increase in the balance occurred, but all of the retained nitrogen seemed to be lost in the following period. In *Russell J.*, an achondroplastic dwarf, no significant change is noted in nitrogen. In the case of calcium it was curious that the urinary excretion, already quite low, disappeared entirely with the use of the hormone, but the balance grew less with increased fecal loss.

Lucille R. Height was 56½ in., weight 96 lb., age 19 years. This child was an inmate of an institution for mental defectives. In addition to her mental difficulty she was dwarfed physically. Roentgenograms of the wrists showed closed epiphyses. An attempt to influence growth antuitrin-G had been followed by glycosuria and for this reason we were permitted to observe her metabolically. Glucose and phosophorus

curves before and during the administration of the hormone showed little effect on the glucose but the phosphorus curve during the time the hormone was administered showed the failure of reciprocal recovery noted in the diabetic. The effect on nitrogen retention was a slight but significant diminution.

TABLE 15

Russell J. Age 10 months. Diet: Klim 80 gm. Dextrimaltose 50 gm. HOH 33 oz.

Dates	NITROGEN			CALCIUM			B.M.R.	R.Q.	Basal Cal.	Wt.	Ht.	Remarks
	Output	Intake	Balance	Output	Intake	Balance						
9/23–27	2.32	3.11	+0.78	0.399	0.731	+0.332				10#	12 oz.	
9/30–10/1	2.77	3.11	+0.34	0.476	0.731	+0.255				10#		
			+0.65			+0.310	Mean Balance					
10/16–19	2.08	3.11	+1.03	0.430	0.731	+0.301				11#	11 oz.	Ant.-G. 1 cc. q other
10/29–11/1	2.41	2.93	+0.51	0.497	0.702	+0.205				12#	13 oz.	day
11/14–17	2.58	3.25	+0.67	0.663	0.757	+0.094				13#	3 oz.	
			+0.74			+0.200	Mean Balance					
11/18–21	2.50	3.20	+0.70	0.479	0.738	+0.259				13#	6 oz.	
12/4–7	2.79	3.14	+0.35	0.560	0.755	+0.195				13#	9 oz.	
12/14–16	2.56	3.04	+0.48	0.478	0.701	+0.223				13#		
			+0.51			+0.226	Mean Balance					

Delores E. Standard Experimental Diet 1-6 15-35-50%

Dates	NITROGEN			B.M.R.	R.Q.	Basal Cal.	Wt.	Ht.	Remarks
	Output	Intake	Balance						
1937									
2/5–8	7.71	9.80	+2.09	−20		920			
2/9–12	8.06	10.04	+1.98	−20		965			
			+1.38	Mean Balance					
2/16–19	7.97	8.34	+0.35	−19		1002			Ant.-G. 3 cc. 2/17–18–19
2/20–22	8.34	10.04	+1.70	−22		962			
2/23–25	9.00	7.67	−1.33	−29		868			
			+0.19	Mean Balance					
Lucille R.									
1938									
3/18–19	10.89	13.20	+2.31						
3/20–22	11.72	11.96	+0.24						
			+1.07	Mean Balance					
3/23–25	13.02	13.20	+0.18	− 2	.85	1133	96	56½	Ant.-G. 5 cc. 3/25
3/26–28	10.94	11.82	+0.88	+ 1	.88	1167	94½	56½	Ant.-G. 5 cc. 26–27
			+0.53	Mean Balance					
3/29–31	12.33	13.20	+0.87						
4/1–3	12.44	13.20	+0.76						
4/4–6	11.98	13.20	+1.22	+ 5	.83	1203	95	56½	
			+0.91	Mean Balance					

Delores E. Age was 11 years, height 53½ in., weight 76 lb. This child was a typical cretin being observed during the administration of, and following the discontinuance of thyroid. It was noted that retention of nitrogen increased paralleling the rise in the B.M.R. with thyroid, and diminished during the fall in metabolism following the omission of thyroid. Antuitrin-G was administered 6 weeks after the discontinuance of thyroid, when the effect of the latter may not have completely worn off. The balances had been steadily declining. A slight rise seemed to occur during the period following the administration of antuitrin-G but the retained nitrogen was promptly lost in the following period.

Russell J. Age was 10 months, weight 12 lb. 12 oz. This was a typical achondro-

plastic dwarf whose study is of some interest aside from its relation to the hormone involved in that there is demonstrated a normal retention of nitrogen and calcium in this condition. Antuitrin-G was administered in 1 cc. doses every other day for one month, a total of 15 cc. The balances cover 12 days of the period during which it was

TABLE 6

1935 Dates	NITROGEN Output	Intake	Balance	B.M.R.	R.Q.	Basal Cal.	Wt.	Remarks
Leonard McC. 205926 Age 9 years								Perthe's Disease
				Diet 2400 Cal. 15-35-50%				
6/12–14	0.42	14.04	+4.63	−21				
6/15–18	7.71	14.04	+6.33					
	Mean Balance		+5.47					
6/19–21	8.17	14.04	+5.87	−18				Ant.-G. 5 cc. 6/19 & 21
6/22–34	7.87	14.04	+6.17					
6/25–27	8.70	14.04	+5.34					
6/28–7/1	8.45	14.04	+5.59					
	Mean Balance		+5.69					
Phyllis S. 197015 Age 12 years Weight 104 lb.								Pott's Disease
				Standard Experimental Diet 1–6 2200 Cal.				
8/14–20	10.95	13.20	+2.25	+10	.79	1522	104	
				+ 1	.78	1440	105¼	
8/21–25	10.16	13.20	+3.04	− 2	.77	1380	104¾	Ant.-G. 5 cc. qd.
8/26–9/5	13.15	13.20	+0.05	+ 6	.81	1502	105¾	
				+ 2	.80	1447	105¼	
Frank S. 246485 Age 9 years								
				Standard Experimental Diet 1–6 1400 Cal				
1/26–31	8.41	9.95	+1.54	− 3	.79	1118		
2/1–4	9.04	9.95	+0.91	± 0	.81	1160		Ant.-G. 1½ cc. 2/1 Ant-G. 3 cc. qd. 2/2–3–4
2/5–6	8.93	9.95	+1.02	−10	.83	1034		
John D. 198577 Born 2–19–29 Ht. 47¾ in. Wt. 45 b. Est. Wt. 53 lb.								
				Standard Experimental Diet 1–6 1500 Cal.				
9/4–11	8.14	8.61	+0.47	− 7	.81	965	45	
				− 5	.88	979	45¼	
9/12–14	8.39	8.59	+0.20	+ 1	.84	1051	46	Ant.-G. 8 cc. qd.
9/15–24	8.05	8.73	+0.68	+ 2	.78	1062	46	
				± 0	.83	1040	45¾	
				+ 2	.82	1068	46	

administered, a 7-day fore-period and an 11-day after-period, but these unlike all others in the series are not continuous because of collection difficulties at this age. A slight increase in nitrogen retention was noted which was lost in the after-period.

Calcium showed a diminished retention during the administration and in the

after-period. This was entirely the result of increased stool calcium, a small urinary output disappearing entirely with the administration of the hormone.

Leonard McC., *Frank S.*, *John D.*, and *Phyllis S.* There are grouped together in table 6, figure 6, the results on nitrogen of 4 cases in which no specifically permanent anabolic effect on nitrogen occurred.

Leonard McC. Age was 9 years, height 54 in., weight 85 lb. This child had been under sporadic observation for a definite osteochondritis for one and a half years before this metabolism observation was made. The nitrogen balance was quite high and the B.M.R. definitely low; nitrogen was not significantly affected by the growth hormone.

Frank S. Age was 9 years, 5 mo., height 50 in., weight 59 lb. This boy was hospitalized because of a tonsillectomy and the observations here reported were made 27 days following the operation. The effect on nitrogen would appear insignificant.

John D. Age was 8 years, 7 mo., height 47¾ in., weight 45 lb. This child had a tonsillectomy on August 16 and these observations were made on September 4. No significant change followed the use of antuitrin-G.

Phyllis S. was an eleven-year-old girl whose menarche occurred 7 months after these studies were done. She was hospitalized because of Pott's disease for which a spinal fusion had been done some months previously. Retention increased following antuitrin-S but all of the retained nitrogen was lost in the after period.

SUMMARY

There are presented observations on the nitrogen balance, and in some instances the calcium balance, in children of the pre- and post-puberty periods following the administration for short periods of antuitrin-G and antuitrin-S. A variety of responses followed—anabolic, katabolic and negative—which might be explained as being conditioned by *a*), the presence or absence of a hormonal defect; *b*, the state of the stores as judged by the control balance and *c*, the age of the child. Subjects included several girls with menstrual difficulties at puberty, 4 dwarfs—a cretin, an achondroplastic and 2 ateleotics, and a number of children who apparently had no endocrine abnormalities. Instances of complete loss of response in the nature of antihormone effect were encountered.

Our thanks are due to Dr. Frank Hartmann who supervised the metabolism determinations, to Dr. O. H. Gaebler who supervised the balance determinations and to Parke, Davis & Co. for the hormone material used.

REFERENCES

1. Maroney, J. W., and J. A. Johnston: *Am. J. Dis. Child.* 54: 29. 1937.
2. Janney, N. W.: *Arch. Int. Med.* 22: 187. 1918.
3. Lee, M. O., and N. K. Schaffer: *J. Nutrition* 7: 337. 1934.
4. Gaebler, O. H.: *J. Exper. Med.* 31: 14. 1933.
5. Collip, J. B., and E. M. Anderson: *Lancet* 1: 76. 1934.
6. Lee, M. O.: The Pituitary Gland. Williams and Wilkins, Baltimore, 1938. p. 215.
7. Pugsley, L. I., and E. M. Anderson: quoted by Lee, *ibid*, p. 206.
8. Teel, H. M., and H. Cushing: *Endocrinology* 14: 157. 1930.
9. Gaebler, O. H.: *Endocrinology* 19: 63. 1935.
10. Johnston, J. A., and J. W. Maroney: *Am. J. Dis. Child.* 51: 1039. 1936.
11. Johnston, J. A., and J. W. Maroney: *J. Pediat.* 12: 563. 1938.

THE EFFECT OF ESTROGENS AS MODIFIED BY ADRENAL INSUFFICIENCY

H. SELYE AND G. MASSON

From the Department of Anatomy, McGill University and the Provincial Veterinary School of Oka

MONTREAL AND QUEBEC, CANADA

RECENTLY Cramer and Horning (1) discussed experiments designed to show close interrelations between the adrenals, the ovaries and the thymus. In agreement with observations on the rat in which estrone and estradiol were used (2, 3, 4), these authors found that in the mouse, estrin (dihydro-progynon) is very toxic if it is administered immediately after adrenalectomy. Furthermore they made the interesting discovery that mice pretreated with estrin for several months are resistant to the toxic effects of estrin even if their adrenals are subsequently removed. This latter observation is in accord with findings (5, 6) indicating that one of the most outstanding features of adrenal insufficiency is a disturbance in adaptation. While adrenalectomized rats are unable to withstand the damage resulting from the first exposure to various drugs, excessive muscular exercise or cold, the animals prove to be quite resistant to such stimuli when pretreated with them prior to the ablation of the suprarenals. It is more difficult to explain why the estrin-pretreated animals of Cramer and Horning (1) survived adrenalectomy better than untreated controls. It must be kept in mind, however, that 13 out of their 24 untreated adrenalectomized control mice survived the operation one month or longer so that the possibility that accessory cortical tissue might have been present in these animals must be considered even though the authors were unable to detect such tissue at autopsy. This consideration appears all the more justified since accessory cortical tissue is very common in the mouse (7, 8) and since mice in which accessory adrenals are not found usually succumb within a few days after suprarenalectomy (7).

The 'brown degeneration' of the adrenals and the atrophy of the thymus observed by Cramer and Horning (1) in estrin-treated mice would not in itself necessarily show any close correlation between the gonads, adrenals and the thymus, since these changes are identical with those produced by any other damaging agent and have therefore been regarded as signs of the 'alarm reaction' (9, 10). It is curious, however, that if an 'alarm reaction' is elicited by non-specific damaging agents in adrenalectomized animals, then thymus atrophy will either not occur at all (11, 12, 13) or if it occurs, it will be negligible as compared with the degree of thymus involution produced by the same agent in the presence of the adrenals (14). The acute involution of the lymph nodes is also prevented or at least decreased following adrenalec-

tomy. Among the large number of agents proven to cause thymus and lymph node involution in normal rats only adrenal cortical extracts (11), estrin (2) and testosterone (4) produced such effects in the absence of the adrenals. It is surprising therefore, that in their experiments on the mouse, Cramer and Horning (1) saw no thymus involution after estrin treatment.

In view of this apparent contradiction, we repeated our experiments both with estrone and with the new synthetic estrogenic substance 4:4′ dihydroxy-α:β-diethylstilbene (diethylstilbestrol). The experiments with estrone showed that in the rat, this substance causes marked thymus and lymph node involution even after suprarenalectomy. Since this is merely a confirmation of our previous work, we shall not discuss these observations here. The experiments with diethylstilbestrol, on the other hand perhaps deserve a short discussion. The high estrogenic activity of this compound was discovered by Dodds et al. (15). It closely resembles naturally occurring estrogens as far as its physiological actions are concerned except that, judged by the dosage required to produce estrus in ovariectomized rats, it is 2.5 times as active as estrone (16). The similarity between the ovarian hormone and this synthetic estrogen is also well shown by the fact that both these substances restore the castrate pituitary to normal (17), cause growth inhibition (18) and gonadal atrophy both in male and in female rats (19). It seemed of interest therefore, to establish whether it would also exhibit the characteristic action on the thymus of the adrenalectomized animal.

EXPERIMENTS

Our experiments were performed on 5 to 7 weeks old female 'hooded' rats weighing 70 to 95 gm. The adrenals were removed through bilateral lumbar incisions in one session. The fat tissue surrounding the gland was also removed in order to make certain that the extirpation is complete. The absence of adrenal remnants and accessory tissue was ascertained at autopsy, as far as this is possible, with the aid of a magnifying lens. For the first experiment 16 rats were used. Eight received a single subcutaneous injection of 5 mg. of diethylstilbestrol[1] dissolved in 1 cc. of olive oil, 24 hours after adrenalectomy. The remaining 8 acted as controls and received 5 mg. of cholesterol in 1 cc. of olive oil subcutaneously the day after their adrenals were removed. We used cholesterol for these control experiments because this substance though structurally related to the estrogenic hormones does not have estrogenic activity.

Within 2 days after the injection, most of the stilbestrol-treated animals became very weak and shaky while the controls remained in good condition. On the third and fourth day after treatment all the stilbestrol injected rats died while among the control animals only one died. A second one, though still alive, was in very bad condition while the remaining 6 were still strong and active. These were killed on the fourth day so that their organ weights would be comparable to those in the control group. It was observed that the average thymus weight in the stilbestrol treated group was 212 mg. In 3 animals of this group, the thymus weight was below 200 mg. The average weight of the 3 iliac lymph nodes [dissected according to the technic described by Selye (11)] was 9.9 mg. The average thymus weight of the

[1] The authors are indebted to Drs. Gregory Stragnell and Erwin Schwenk of the Schering Company for kindly supplying this material.

controls was 352 mg., the smallest gland observed being 270 mg. The average weight of the iliac lymph nodes in this group was 16.6 mg.

In another group of 8 adrenalectomized animals, we introduced 15 mg. of diethylstilbestrol in crystalline form into the peritoneum immediately after adrenalectomy. All but 2 of these animals died within 2 to 4 days after the operation. The average weight of their thymus glands was 157 mg. and 2 glands weighed only 75 and 68 mg. respectively. It should be emphasized that there was no close correlation between the length of survival and the intensity of the thymus involution. The average weight of the iliac lymph nodes was 14.5 mg. in this group. It appears obvious from the foregoing observations that diethylstilbestrol resembles estrone and other estrogenic substances in as far as its effect on the thymus and lymph nodes is concerned and that like testosterone and the natural estrogens, this synthetic estrogenic compound though not very toxic for the normal individual is extremely toxic in the absence of the adrenals.[2]

DISCUSSION

In view of the fact that among all substances hitherto examined only adrenal cortical extract, testosterone and estrogens were found to cause thymus involution in the absence of the adrenals, it appears justified to suspect some close physiological correlation between adrenals, thymus and sex hormones. Such a view receives further support from the experiments reported in this communication which show that a synthetic estrogen exerts the same effect on the thymus as the sterols produced by the adrenals and gonads although chemically it is entirely different from them.

Cramer and Horning (1) claimed that the typical actions of estrogens are decreased or completely prevented by adrenalectomy. In order to explain this finding, they state "There are the alternative possibilities that the estrogenic ovarian hormone requires for its activity a preliminary transformation in the adrenal gland, or that it needs the simultaneous presence of the synergic adrenal hormone." It is felt that neither of these two theories could explain the fact that adrenalectomized rats may show normal estrous cycles if treated with sodium chloride (20). Another observation difficult to explain on the basis of these assumptions is that all the adrenalectomized rats of our present experimental series whether treated with estrone or diethylstilbestrol showed full uterine estrus and vaginal cornification. Cramer and Horning (1) do not mention whether they examined normal mice treated with estrin for the same length of time as the adrenalectomized estrin-treated animals. Since the latter received pretreatment with estrin for several months prior to the suprarenalectomy, one wonders whether a certain adaptation to estrin could not have occurred. The suspicion that such an adaptation is not quite impossible appears justified perhaps in view of the fact that following a period of pretreatment, estrin loses its corpus luteum forming effect in the rat (21). The fact that the unpretreated adrenalecto-

[2] In comparing the toxicity of the naturally occurring estrogens with that of diethylstilbestrol in normal mice, it was found (23) that weight for weight, the latter compound is more toxic than either estradiol or estrone. It should be kept in mind that diethylstilbestrol was shown to be only 2.5 times (16) while estradiol is about 20 times as active as estrone (24) so that if compared on the basis of their biological activity, diethylstilbestrol would appear to be relatively even more toxic than what might be surmised on a weight for weight basis.

mized mice in Cramer and Horning's (1) series were killed by estrin while the pretreated ones were not damaged by this hormone also appears to indicate that a certain degree of adaptation to estrin is possible. If this supposition should prove correct, it would be easy to explain the absence of thymus involution in the adrenalectomized mice which were pretreated with estrin. Boinet (22) has shown in 1899 and Jaffe (23) and Marine et al. (24) confirmed that adrenalectomy in itself tends to enlarge the thymus. This we believe is not a true hypertrophy but merely an enlargement to the maximum size which is ordinarily obtained only just before puberty (11). Cramer and Horning (1) also confirmed the occurrence of thymus enlargement following suprarenalectomy and this factor may have helped to assure a relatively large thymus in animals in which the action of estrin was perhaps already decreased by prolonged pretreatment. In any case, we feel that the literature quoted and the experiments reported in this communication show beyond doubt that the presence of adrenal tissue is not essential for the effect of estrogens on the thymus.

SUMMARY

While any damaging agent may cause thymus involution in the normal organism, no such effect is seen in the absence of adrenal tissue.

Since adrenal cortical extracts cause thymus involution even in adrenalectomized animals, it seems likely that the action of non-specific damaging agents on the thymus is an indirect one mediated by the adrenal cortex. Under the influence of damaging agents, the adrenal cortex shows histological signs of increased activity and it is assumed that the thymus involution which occurs at this time, is due to an excess production of adrenal cortical hormone.

Apart from adrenal cortical extracts, only estrone, estradiol, diethylstilbestrol and testosterone were found to cause thymus involution in the absence of the adrenals.

Diethylstilbestrol also resembles the adrenal cortical and gonadal hormones in causing lymph node involution in the absence of adrenal tissue.

Like the naturally occurring estrogens and testosterone, diethylstilbestrol though relatively innocuous for normal animals is highly toxic for adrenalectomized rats.

The estrogenic effect of estrone and diethylstilbestrol as judged by their action on the uterus and vagina of the rat is not prevented by adrenalectomy.

The chemical structure of diethylstilbestrol is entirely different from that of the estrogenic hormones, yet its effect on the thymus and the lymph nodes of the adrenalectomized rat is the same as that of naturally occurring estrogens or testosterone. It appears therefore, that the specific action of gonadal hormones on the thymus is not necessarily due to their chemical structure but is in some way connected with their sex stimulating effect.

REFERENCES

1. Cramer, W., and E. S. Horning: *Lancet* 1: 192. 1939.
2. Selye H., C. M. Harlow und J. B. Collip: *Endokrinol.* 18: 81. 1936.
3. Selye, H.: *Am. J. Physiol.* 122: 347. 1938.

4. SCHACHER, J., J. S. L. BROWNE AND H. SELYE: *Proc. Soc. Exper. Biol. & Med.* 36: 488. 1937.
5. SELYE, H.: *Arch. Internat. Pharm. e. de Thér.* 55: 431. 1937.
6. SELYE, H.: *Science* 85: 247. 1937.
7. MASUI, K.: *Endokrinol.* 2: 19. 1928.
8. WHITEHEAD, R.: *J. Pathol. & Bact.* 35: 415. 1932.
9. SELYE, H.: *Nature* 138: 32. 1936.
10. SELYE, H.: *Endocrinology* 21: 169. 1937.
11. SELYE, H.: *Brit. J. Exper. Pathol.* 17: 234. 1936.
12. LEBLOND, C. P., AND G. SEGAL: *Compt. rend. Soc. de Biol.* 128: 995. 1938.
13. SEGAL, G., AND C. P. LEBLOND: *Compt. rend. Soc. de Biol.* 129: 279. 1938.
14. LEBLOND, C. P., AND G. SEGAL: *Compt. rend. Soc. de Biol.* 129: 838. 1938.
15. DODDS, E. C., L. GOLDBERG, W. LAWSON AND R. ROBINSON: *Nature* 141: 247. 1938.
16. DODDS, E. C., W. LAWSON AND R. L. NOBLE: *Lancet* 1: 1389. 1938.
17. WOLFE, J. M.: *Am. J. Physiol.* 115: 665. 1936.
18. NOBLE, R. L.: *Lancet* 2: 192. 1938.
19. NOBLE, R. L.: *J. Physiol.* 94: 177. 1938.
20. KUTZ, R. L., T. MCKEOWN AND H. SELYE: *Proc. Soc. Exper. Biol. & Med.* 32: 331. 1934.
21. SELYE, H., J. B. COLLIP AND D. L. THOMSON: *Proc. Soc. Exper. Biol. & Med.* 32: 1377. 1935.
22. BOINET, E.: *Compt. rend. Soc. de Biol.* 51: 671, 673. 1899.
23. SELYE, H.: *Canad. Med. Assoc. J.* 1939 (in press).
24. LAUSON, H. D., C. G. HELLER, J. B. GOLDEN AND E. L. SEVRINGHAUS: *Endocrinology* 24: 35. 1939.

A METHOD OF ASSAY FOR THE FAT METABOLISM HORMONE OF THE ANTERIOR PITUITARY[1]

O. B. HOUCHIN AND C. W. TURNER
WITH THE TECHNICAL ASSISTANCE OF W. D. COOPER
From the Department of Dairy Husbandry, University of Missouri
COLUMBIA, MISSOURI

FOR THE FURTHER chemical purification of the fat metabolism hormone of the anterior pituitary and study of its physiology, quantitative assay methods become important. In the early studies of this factor, its influence on fat catabolism as measured by ketonemia and ketonuria was described. Later the fatty infiltration of the liver was observed to follow the injection of AP extracts. On the basis of this observation, Campbell (1) has recently suggested an assay technique using the mouse. Recently, Houchin and Turner (2) reported that single injections of an AP extract depressed the blood fat of rabbits about 36% in from 6 to 8 hours. As our interest in this hormone centers about its relation to fat secretion by the mammary gland (that is milk fat precursors in blood) it seemed desirable to develop an assay method dependent upon blood lipid changes.

The rabbit would serve very well as an assay animal except for the rather large amount of hormone required to induce a response. The blood lipids of the female guinea pig were observed to be quite sensitive so it was decided to explore the usefulness of this animal in the assay of the hormone.

For the determination of the blood fat, the rapid method described by Allen (3) was found satisfactory. He has shown that this method includes the neutral fats, sterols, sterol esters and lipochromes which were designated as blood fats. Phospholipids are not included. Further, it was shown that this method compared favorably with other methods of blood fat determinations (Bloor's and Boyd's method) when phospholipids are excluded.

To determine whether alterations in blood fat are correlated with the phenomena of liver fat infiltration, determinations of liver fat were made on the test animals.

EXPERIMENTAL

Normal blood and liver fat values for the guinea pig. For the determination of the normal blood fat value of young female guinea pigs, a total of 34 animals in a good nutritive state were killed at various times during the day and

[1] This study has been aided in part by a grant from the Committee on Research in Endocrinology of the National Research Council.
Contribution from the Department of Dairy Husbandry, Missouri Agricultural Experiment Station, Journal Series, No. 598.

night. The oxalated blood was centrifuged and the plasma fat determined by the method of Allen (3). The body weight ranged from 150 to 240 gm. with an average of 191.2 gm. The plasma fat varied from 30 to 130 mg.% with a mean of 60.44. The body weight appeared not to influence the plasma fat as the mean fat content of the animals under 200 gm. was practically the same as the group over 200 gm. Withholding food for 24 hours previous also appeared to be without effect as 5 animals so treated had an average blood fat content of 59 mg.%.

Best and Campbell (4) reported that the liver of normal fasted guinea pigs weighing about 300 gm. contained 136 mg. of fat per 100 gm. of initial body weight or 4.3% fat. For comparison, data on a group of 26 female guinea pigs not previously starved were obtained in our laboratory. The range of liver fat from 32.6 to 276 mg. was rather large, yet the mean value was 132.6 mg.% of fat per 100 gm. body weight. The average liver weight was 6.006 gm. and contained 251.7 mg. or 4.19% of fat.

Procedure. Well nourished female guinea pigs weighing from 160 to 210 gm. were used. The temperature of the animal room was kept at between 70° to 80° F. as it is suspected that fluctuations in temperature may influence the blood fat content. The 'initial extract' prepared from cattle anterior pituitaries by Bergman and Turner (5) was injected intraperitoneally. Blood samples were then obtained at varying intervals by killing the animals, and analyzed for plasma fat. The livers were excised, freed of blood and tissue, and weighed. The liver was diced into small pieces and the fat extracted with an alcohol-ether mixture (3:1). The alcohol-soluble non-fatty material was then precipitated and the fat weighed.

TABLE 1. EFFECT OF AP EXTRACTS UPON THE BLOOD PLASMA (ALLEN METHOD) AFTER VARYING INTERVALS

Amount of hormone injected	Blood fat at various intervals following injection						
	4 hr.	6 hr.	8 hr.	10 hr.	12 hr.	24 hr.	26 hr.
	mg. %	mg. %	mg. %	mg. %	mg. %	mg. %	mg. %
20.0 mg.	18	0, 0	10, 20, 75	16.0	15, 70	2500,[1] 60, 40	240,[1] 110
20.0 mg.	15	5, 0	45, 20, 0	25, 48	270,[1] 30	20, 0, 30	15, 85, 30
20.0 mg.	9	15, 8	240,[1] 20, 30	65, 60	15, 55	105, 7	50, 20
20.0 mg.	2	10, 4	25, 35, 40	10	22, 40	50, 35, 0, 20	
20.0 mg.		5, 8	40, 60			30, 8	
20.0 mg.		20					
Ave.	11.0	6.8	31.5	39.8	35.3	31.8	51.7

[1] Excluded from average.

Observations. To determine the most satisfactory time interval to obtain the maximum depression of the plasma fat, 20 mg. of extract was injected into each of 9 groups of animals which were then killed at intervals ranging from 4 to 26 hours (table 1). From these observations it was concluded that a 6-hour interval would be most satisfactory. Further it was noted that a 50% depression of the fat could be obtained readily when using the Allen method of plasma fat determination.

When the amount of AP extract injected was varied, it was observed that the injection of 10 mg. caused a decrease of about 50% in blood plasma fat (table 2). Larger amounts depressed the fat very materially (to 6.77 mg.%). At the 10 mg. level, a group of 5 animals were killed after 4 hours. The average blood fat was 37.0 mg.%. Similarly, a group of 4 animals killed after 24

TABLE 2. EFFECT OF INCREASING AMOUNTS OF AP EXTRACT UPON THE BLOOD PLASMA FAT (ALLEN METHOD)

Interval between injection and killing	Blood fat following anterior pituitary extract					
	5.0 mg.	8.0 mg.	10.0 mg.	12.5 mg.	15.0 mg.	20.0 mg.[1]
hr.	mg. %	mg. %	mg. %	mg. %	mg. %	mg. %
6	70, 110	35, 70	15, 0, 15, 8, 60	30, 15, 50	40, 20	0, 0, 5, 0, 15, 8, 10
6	50, 140	30, 130	30, 50, 30, 30,	12, 15, 12	10, 20	4, 5, 8, 20
6	27, 28	90, 23	25, 60	21		
6	45, 50	60				
Ave.	65.00	61.14	29.30	22.14	22.50	6.77

[1] Data from table 1 for group.

hours showed average blood plasma fat of 42.5 mg.%. These values are in line with previous observations (table 1).

Observations on the fat content of the liver of guinea pigs injected with

TABLE 3. RELATION BETWEEN BLOOD PLASMA FAT AND LIVER FAT FOLLOWING VARYING AMOUNTS OF AP EXTRACT

Amount of APE injected	Interval between injection and killing	Blood Fat[1]		Analysis of Liver				
		No. animals	Average	No. animals	Ave. weight	Ave. weight of fat	Ave. % fat	Ave. fat 100 gm. body wt.
mg.	hr.		mg. %		gm.	mg.		mg.
Normal Control		34	60.4	26	6.01	251.7	4.19	132.6
5	6	8	65.0	7	6.65	482.7	7.25	260.4
8	6	7	61.1	6	6.41	415.2	6.46	233.5
10	4	5	37.0	5	6.47	421.9	6.62	237.2
10	6	11	29.3	25	7.09	479.4	6.98	259.9
10	24	4	42.5	4	6.02	403.8	6.67	255.3
12.5	6	7	22.1	7	6.33	477.7	7.65	254.9
15.0	6	4	22.5	4	6.61	516.5	7.88	286.5
20.0	4	4	11.0	3	5.86	433.7	7.48	245.5
20.0	6	11	6.8	11	6.88	530.4	7.71	293.3
20.0	8	13	31.5	10	6.51	513.4	7.92	295.7
20.0	10	6	39.8	5	18.45	670.0	17.93	346.7
20.0	12	7	35.3	7	7.43	624.0	8.40	407.4
20.0	24	14	31.8	14	5.77	401.7	6.10	221.4
20.0	26	6	51.7	2	7.78	826.7	10.60	258.7

[1] By Allen method (3).

varying amounts of extract and killed after varying time intervals are presented in table 3. It is interesting to note that the injection of 5 and 8 mg. of extract caused considerable fatty infiltration of the liver yet had no ap-

parent effect in lowering the blood plasma fat.[2] It appears that the injection of larger amounts of extract and longer intervals did not appreciably increase the fat content of the liver. These observations suggest that the production of fatty livers may respond to smaller amounts of hormone yet the alteration of the blood fat content is more sensitive to variations in dosage.

Guinea pig unit of fat metabolism hormone. These data form the basis for the formulation of a tentative procedure for the biological assay of the fat metabolism hormone using the depression of the blood plasma fat as a criterion of response. It is proposed that female guinea pigs in good thriving physical condition weighing from 160 to 200 gm. be used. They should be maintained at a fairly uniform temperature such as 70 to 80° F. The pituitary extract, in the form of a suspension, is injected intraperitoneally in the morning and the animals killed 6 hours later. The plasma fat is determined by the method of Allen (3). One unit of hormone is defined as the minimum amount of extract which will depress the plasma fat of at least 6 animals, on the average, to about 30 mg.% or 50% of the normal. Infrequently an animal may be found to be unresponsive to the extract. When their blood plasma fat values are above the normal, it is our opinion that one is justified in discarding such values.

To determine the reproducibility of successive assays at the unit dosage, a number of groups of animals were injected with the same extract at the 10 mg. level as this amount showed a reduction of approximately 50% in blood plasma fat (see table 2).

	mg.%	*Ave. mg.%*	*Fat Reduction%*
Group I	15, 0, 15, 8, 60, 30, 50, 30, 30, 25, 60	29.3 (11 animals)	51.5
Group II	18, 15, 60, 20, 25, 35	29.0 (6 animals)	52.0
Group III	20, 15, 20, 20, 20, 45	23.4 (6 animals)	61.3
Group IV	15, 35, 60, 50, 15, 10	30.9 (6 animals)	48.8
Group V	8, 30, 20, 40, 25, 38	27.0 (6 animals)	55.3
Group VI	25, 15, 30, 25, 48, 25	28.0 (6 animals)	53.6

The average plasma fat of the entire group of 41 animals was 28.1 mg.% or an average decline of 53.5%. Only 4 animals in this group had values equal to the normal average. One or two additional animals were excluded for exceeding the normal value. In only one assay was a tolerance of ±10% exceeded. Greater accuracy could be obtained by increasing the number in the group.

SUMMARY

A study is reported on the use of well nourished female guinea pigs in the biological assay of the fat metabolism hormone of the anterior pituitary, using a depression of the plasma fat as the criterion of response.

A group of 34 normal females weighing from 150 to 250 gm. showed an average plasma fat content of 60.44 mg.% as determined by the Allen method. The livers of 26 animals weighed an average of 6.01 gm. and contained 251.7 mg. (132.6 mg.% per 100 gm. body weight) or 4.19% of fat.

[2] Attention should be called to the fact that the method of liver fat determination includes all ether-soluble fats including phospholipids which are excluded in the blood fat determination.

Upon the injection of varying amounts of AP extract the plasma fat of the guinea pig was depressed to amounts varying with the amount injected. The maximum depression was reached about 6 hours after intraperitoneal injection of the extract. At the 10 mg. level, a depression of 50% in blood plasma fat was produced. Associated with the depression in blood plasma fat was a fatty infiltration of the liver. Our data suggest that smaller amounts of extract influence the fat content of the liver than of the blood plasma. However, the blood plasma fat appears to be more sensitive to variations in the amount of extract injected than does the liver fat although this determination includes phospholipids.

One unit of fat metabolism hormone is defined as the minimum amount of extract which will depress the plasma fat of at least 6 animals, on the average, to about 50% of the normal (normal about 60 mg.%), or to about 30 mg.%.

To determine the reproducibility of successive assays at the unit dosage, 6 groups of animals were each injected with 10 mg. of the same extract. Of these only one group exceeded a tolerance of ±10%.

The sample of cattle pituitary extract (an initial extract) contained about 100 units per gm. of dried extract (10 mg. per unit).

REFERENCES

1. CAMPBELL, J.: *Endocrinology* 23: 692. 1938.
2. HOUCHIN, O. B., AND C. W. TURNER: *Endocrinology* 24: 638. 1939.
3. ALLEN, N. N.: *Proc. Soc. Exper. Biol. & Med.* 31: 991. 1933; *Minn. Agr. Exp. Sta. Bull.* No. 130, 1938.
4. BEST, C. H., AND J. CAMPBELL:. *J Physiol.* 92: 91. 1938.
5. BERGMAN, A. J., AND C. W. TURNER: *J. Biol. Chem.* 123: 471. 1938.

CREATINE-CREATININE METABOLISM AND THE HORMONES II. PROGESTIN AND ANTUITRIN-T[1]

A. LINK KOVEN AND HOWARD H. BEARD
From the Department of Biochemistry, School of Medicine, Louisiana State University
NEW ORLEANS, LOUISIANA

IN THE first study in this series (1) results were published showing that castration in both sexes of young adult white rats resulted in creatinuria which was more intense in the female than in the male. The injection of anterior pituitary hormones (G, T and S), theelin, follutein, and testosterone propionate, caused increases in muscle creatin and in creatin excretion accompanied by decreases in creatinine excretion. The injection of all these hormones, with the exception of antuitrin-S and T and of follutein, caused a more significant increase in the total amount of creatine excreted in the castrated rat than in the same rat before castration. A possible synergistic action of the hormones in the body upon creatine and creatinine metabolism was suggested.

In the present study progestin and antuitrin-T, and theelol with antuitrin-S, were injected into normal and castrated female rats. The experimental procedures were given in our first paper of this series (1). The control values for creatine and creatinine excretion were averaged for 5 2-day periods before injections of the hormones were made.

RESULTS

Urine studies; antuitrin-T. The injection of 1 to 3 cc. of this hormone into normal rats 1 to 3 caused slight increases in creatine excretion, which was more marked with the 3 cc. dose (fig. 1). Very significant increases in creatinine excretion occurred. Castration in these animals caused significant increases in both creatine and creatinine excretion. The injection of 1 to 3 cc. of antuitrin-T in the castrated animals caused a larger creatinine excretion with the 1 cc. dose than when 2 or 3 cc. was injected. With 1 and 2 cc. doses the creatinuria was, therefore, more intense in the castrated as compared with the normal animals.

Progestin (commercial). The injection of 5 to 15 U of this preparation into normal animals Nos. 4 to 6 caused slight increases in the creatinuria. In all cases there was an initial increase followed by a decrease in creatinine excretion (fig. 2). From the results of other studies we believe that this retained creatinine was transformed into creatine, the excess of which was then ex-

[1] We wish to thank Dr. Willard M. Allen of the University of Rochester for the crystalline progesterone and Dr. Oliver Kamm of Parke, Davis & Co. for the other hormone preparations used in these studies.

creted into the urine (2). The creatinuria in the castrates with and without injection of progestin was much greater than in the normal animals.

Progesterone (crystalline). This preparation also caused large increases in creatine excretion, especially in the castrated animal (fig. 3). The doses of

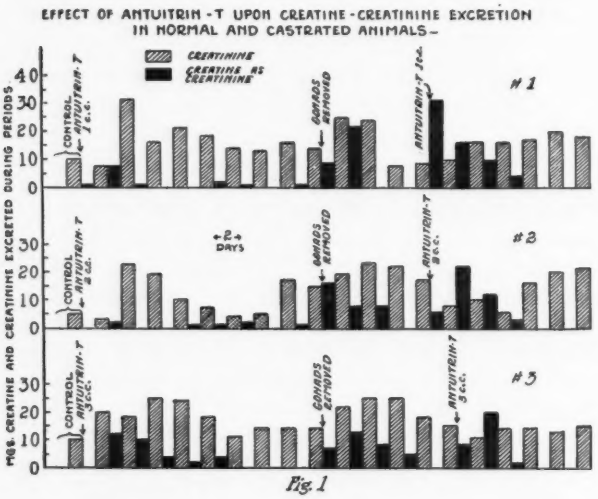


Fig. 1

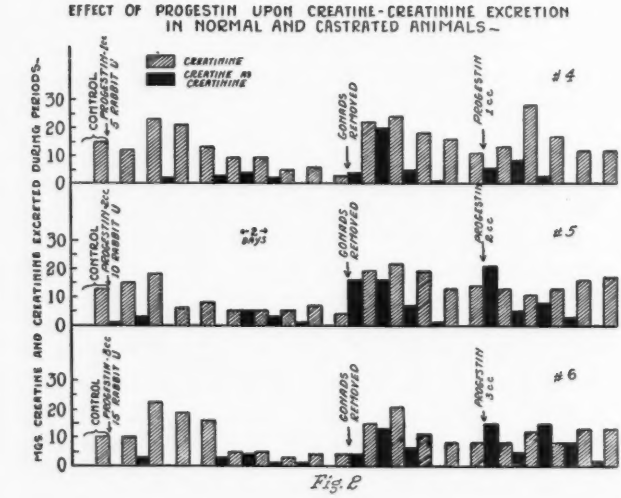


Fig. 2

the commercial and crystalline preparation were very different and comparisons of the creatinuria obtained are, therefore, not justified.

Theelol and antuitrin-S. Schrire and Zwarenstein (3) advanced the theory that the increased creatinine excretion after castration in rabbits was due to

overactivity of the anterior pituitary, which, however, is inhibited by injections of the sex hormones. To test the possibility that the female sex hormone itself may prevent the excretion of creatinine we injected simultaneously 100 U of theelol and 200 U of antuitrin-S in both normal and castrate

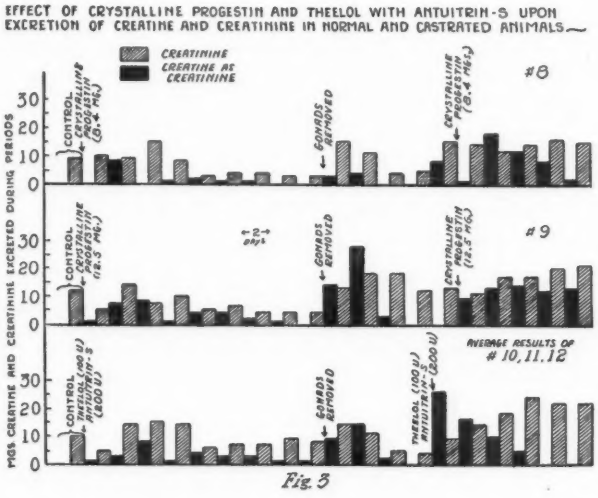

EFFECT OF CRYSTALLINE PROGESTIN AND THEELOL WITH ANTUITRIN-S UPON EXCRETION OF CREATINE AND CREATININE IN NORMAL AND CASTRATED ANIMALS
CREATININE
CREATINE AS CREATININE
#8
#9
CONTROL
GONADS REMOVED
CRYSTALLINE PROGESTIN (8.4 MG.)
CRYSTALLINE PROGESTIN (12.5 MG.)
THEELOL (100 U.) ANTUITRIN-S (200 U.)
AVERAGE RESULTS OF #10, 11, 12
MGS. CREATINE AND CREATININE EXCRETED DURING PERIODS

Fig. 3

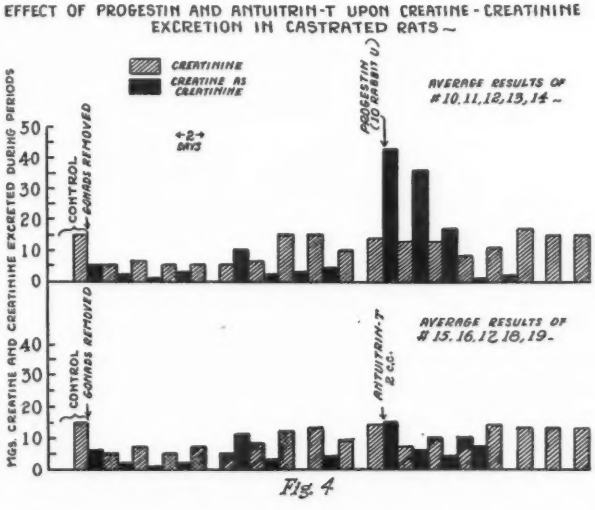

EFFECT OF PROGESTIN AND ANTUITRIN-T UPON CREATINE - CREATININE EXCRETION IN CASTRATED RATS
CREATININE
CREATINE AS CREATININE
PROGESTIN (10 RABBIT U.)
AVERAGE RESULTS OF #10, 11, 12, 13, 14
AVERAGE RESULTS OF #15, 16, 17, 18, 19
ANTUITRIN-T 2 C.C.
CONTROL
GONADS REMOVED
MGS. CREATINE AND CREATININE EXCRETED DURING PERIODS

Fig. 4

rats (fig. 3). There was no increase of creatinine excretion in normal rats after injection of these hormones, but an increased creatine and creatinine excretion under these conditions was observed in the same animals after castration.

Progestin and antuitrin-T. More information on the injection of these hormones in the castrate animal was desired. Ten RB.U. of progestin gave very large increases in creatine excretion in the 5 rats studied (fig. 4). The results with antuitrin-T in another 5 animals were about the same as those shown in figure 1.

TABLE 1. EFFECT OF PROGESTIN AND ANTUITRIN-T UPON THE CREATINE CONTENT OF THE MUSCLES OF NORMAL AND CASTRATE RATS

Hormone	Amount injected	Type of animal	No. of animals	Muscle creatine in %			Experiment
	cc.			*Max.*	*Min.*	*Av.*	*days*
Controls		Normal	20	0.44	0.37	0.42	2
Progestin	2	Normal	4	0.43	0.40	0.42	2
Progestin	2	Castrate	4	0.46	0.44	0.45	2
Antuitrin-T	2	Normal	4	0.47	0.40	0.44	2
Antuitrin-T	2	Castrate	4	0.45	0.40	0.44	2
Controls		Castrate	11	0.45	0.40	0.42	96

TABLE 2. EFFECT OF CASTRATION UPON TISSUE CREATINE IN THE RAT

Type of animal	No. of animals	Creatine Content of				Experiment
			Muscles	Liver	Kidney	Heart	
			%	%	%	%	*days*
Controls	15	Max.	0.44	0.08	0.16	0.26	2
		Min.	0.39	0.06	0.08	0.19	
		Av.	0.41	0.07	0.12	0.21	
Castrates	11	Max.	0.45	0.06	0.10	0.24	96
		Min.	0.40	0.04	0.06	0.16	
		Av.	0.42	0.05	0.08	0.21	

Tissue studies. The results listed in table 1 show that the injection of 2 cc. of progestin or antuitrin-T in normal and castrate rats gave no increased creatine formation in the muscles. Those in table 2 likewise show that castration does not affect the concentration of creatine in the muscles, liver, kidney and heart in the rat.

DISCUSSION

In our previous study (1) it was shown that parenteral injection of the male and female sex hormones and anterior pituitary extract (G, T, and S) caused large and significant increases in muscle creatine. It was, therefore, of interest to observe in the present studies that antuitrin-T did not increase the concentration of muscle creatine. The results obtained with antuitrin-G and S and the male and female sex hormones on creatine metabolism confirm the prevailing view that these hormones are related to muscle metabolism but that progestin and antuitrin-T are not.

It is also well known that the feeding of thyroid tissue or injections of thyroxin induces a creatinuria in man and mammals. The same is true of exophthalmic goiter in man. Pugsley, Anderson and Collip (4) gave a brief

review of the literature on the subject. These investigators obtained a creatinuria in dog and rat after administering desiccated thyroid or thyrotropic hormone. Schrire (5) observed a marked increase in both creatine and creatinine excretion in 9 cases of acromegaly. The injection of the thyrotropic hormone also caused a large creatinuria in normal subjects. Buhler (6) stated that the thyrotropic hormone caused an increased elimination of creatine in dogs, rabbits and monkeys, and Schnittenhelm and Buhler (7) observed the same effect from this hormone in a case of myxedema. Osada (8) observed a decrease in the creatine content of the skeletal muscle of the rabbit after oral administration of corpus luteum powder and after oöphorectomy.

The results obtained by Zwarenstein and his coworkers (3) in the rabbit between creatine-creatinine metabolism and the endocrines are, in some instances, opposite to the effect that we have obtained in rats. Among some of the reasons for this confused state of affairs may be mentioned: the relation of creatine and creatinine to the endocrines in the rabbit may be different from that in the rat; these authors studied the creatine excretion in only a few instances; they believe that creatine is changed into creatinine, which may be true for the rabbit, but not for the rat or man (2). Further discussion of the question would be unprofitable at this time.

At the present time it is very difficult to attempt to explain our results of hormone injections upon creatine-creatinine metabolism. However, we suggest three possibilities for consideration, first, a direct action of the hormone injected, second, an indirect action of the hormone injected through another hormone, or third, the effect of the hormone injected upon water and sodium metabolism.

While all three of these suggestions are attractive, the last is particularly so. Work in our laboratory has just been completed showing that creatinine is changed into creatine in the muscles and stimulates enormously the excretion of both creatine and creatinine in the urine (2). Further studies have shown that the injection of large quantities of physiological saline into young rats causes an increased creatine excretion which is intensified if creatinine is injected simultaneously. No increases in tissue creatine occur under these conditions until very large doses of hypertonic saline are injected. Thus it is evident that there is a close relation between water and creatine metabolism.

Of much interest in this connection is the recent report by Harrop (9) who injected several crystalline sex hormones and noted their 'sodium retaining effects.' His results were as follows in dog units: estradiol, 700; progesterone, 400; estrone, 200; pregnandiol, 140; testosterone, 80; testosterone propionic acid ester, 25. In our experiments injection of progesterone caused a larger creatinuria than that produced by the other sex hormones.

It is, therefore, possible that the creatinuria which we have observed in the normal and castrated rat after injection of the sex hormones may be due, in part at least, to a retention of sodium, which results in a retention of water in the body. This favors the creatinine to creatine transformation, the excess of the latter being then excreted into the urine.

SUMMARY

Commercial progestin, crystalline progesterone and antuitrin-T were injected into young rats before and after castration to study their effects upon creatine-creatinine metabolism.

Castration leads to a temporary creatinuria, but does not affect the concentration of tissue creatine in the rat. Likewise the injection of progestin or antuitrin-T does not change the concentration of muscle creatine. These hormones give a more intense creatinuria in the castrate as compared to the normal animal.

Progestin causes an initial increase followed by a decrease in creatinine excretion in normal animals which is accompanied by an increase in creatine excretion. Antuitrin-T causes an increase in the excretion of both creatine and creatinine in the normal animal.

A possible explanation is given of the effect of the sex hormones on creatine-creatinine metabolism.

REFERENCES

1. Pizzolato, P., and H. H. Beard: *Endocrinology* 24: 358. 1939.
2. Jacobs, E. J., and H. H. Beard: In press.
3. Schrire, I., and H. Zwarenstein: *Biochem. J.* 26: 118. 1932; 27: 1337. 1933; 28: 356. 1934.
4. Pugsley, L. I., E. M. Anderson and J. B. Collip. *Biochem. J.* 28: 1135. 1934.
5. Schrire, I.: *Quart. J. Med.* 6: 17. 1937.
6. Buhler, F.: *Ztschr. f.d. ges. exper. Med.* 96: 821. 1935.
7. Schnittenhelm, A., and F. Buhler: *Ztschr. f.d. ges. exper. Med.* 95: 206. 1935.
8. Osada, S.: *Folia Endocrinol.* 11: 21. 1935.
9. Harrop, G. A.: *Cold Spring Harbor Symposia Quant. Biol.* 5: 375. 1937.

SURVIVAL, STRUCTURE AND FUNCTION OF PITUITARY GRAFTS IN UNTREATED RATS AND IN RATS INJECTED WITH ESTROGEN[1,2]

DORIS PHELPS, E. T. ELLISON AND JOHN C. BURCH

From the Department of Obstetrics and Gynecology, Vanderbilt University School of Medicine

NASHVILLE, TENNESSEE

IT HAS BEEN demonstrated by several investigators that anterior pituitary tissue can be successfully grafted and some information concerning the physiologic activity of such grafts has been obtained (1–11). Schweizer, Charipper and Haterius (1) found that grafts in the anterior chamber of the eye stimulated follicle growth but not luteinization in guinea pigs. Greep (2) observed normal reproductive function in hypophysectomized rats bearing grafts in the sella turcica. May (3) reported similar observations in rats with grafts in the anterior chamber of the eye. These results seem to indicate that the nervous system participates in the production of luteinization in guinea pigs but not in rats. However, Greep's results (2) are open to question because of the site of the grafts, and May (3) described the findings in only 2 animals.

Martins (4) and Desclin and Grégoire (5) found that anterior lobe grafts in non-hypophysectomized rats injected with estrogen showed histologic changes similar to those observed in the pituitaries of the hosts. These results seem to indicate that, with respect to its influence upon structure, estrogen acts directly upon the anterior lobe and not indirectly through the nervous system. On the other hand, the structural changes which occur regularly in the anterior lobe after castration and which can be prevented or corrected by the administration of gonad hormones, have not been observed to occur in pituitary grafts after castration of the hosts (4–6). This would seem to indicate some influence of the nervous system upon the structure of the anterior lobe.

Since estrogen influences both the structure and the function of the anterior lobe, it seemed possible that further investigation of the influence of this hormone upon pituitary grafts might afford additional information concerning the interrelationships which affect the anterior lobe. In the present study, the influence of estrogen upon the survival of pituitary grafts was investigated and observations on the structure and function of such grafts in untreated hosts and in hosts injected with estrogen were made.

[1] Read at the 23rd annual meeting of the Association for the Study of Internal Secretions, St. Louis, May, 1939.

[2] This work has been aided by a grant to Vanderbilt University School of Medicine from the Division of Medical Sciences of the Rockefeller Foundation.

MATERIAL AND METHODS

In 46 experiments, pituitaries from mature male or female rats were grafted into the thigh muscles of mature female rats. As determined by careful check at autopsy, 23 of the hosts were completely hypophysectomized and 23 were partially hypophysectomized. Albino and pied rats from inbred strains reared in our own laboratory were used, host and donor being from the same strain in each case. Hypophysectomy and implantation of the graft were performed at one operation. Donors were killed by exsanguination under ether and the pituitaries were carefully removed and implanted intact. Each host received one pituitary. Nineteen of the hosts were not injected during the experiment and 27 were injected with 200 R.U. of estrogen[3] daily, beginning 16 to 20 hours after operation in 16 experiments, 3 to 25 days after operation in 11 experiments. The animals were weighed every other day and vaginal smears were made daily throughout each experiment. Autopsy was performed at intervals varying from 5 to 60 days after operation (tables 3 and 4). The ovaries and adrenals and, in some experiments the kidneys, were weighed at autopsy. The ovaries, uteri, vaginae, adrenals and 7 of the grafts were fixed in Bouin's solution and stained with hematoxylin and eosin. The remaining grafts were prepared for microscopic study by the method of Cleveland and Wolfe (12). The ovaries and grafts were sectioned serially.

RESULTS AND DISCUSSION

Survival of grafts. Nine surviving grafts were recovered in 10 cases in which 5 to 10 days elapsed between implantation and autopsy. In longer experiments, survival was better in injected than in uninjected hosts, regardless of the sex of the donors and better in the case of grafts from female than from male donors, regardless of the treatment of the hosts (table 1).

Structure of grafts. Grafts recovered from uninjected hosts 20 to 60 days after implantation were smaller than normal. Those from injected hosts were slightly larger than normal adult pituitaries. The size was proportional to the length of the injection period but in no case did it approximate the size attained by pituitaries in situ in mature rats similarly injected (13, 14).

Anterior lobe. The tissue in the central portion was degenerating in the grafts removed 5 days after implantation. The cells in this area were chiefly chromophiles with pyknotic nuclei and no discernible cell membranes. Peripherally, there was a narrow border of normal tissue (fig. 1) in which were large vascular spaces containing normal erythrocytes. In the 10 to 20-day specimens, the peripheral normal tissue surrounded a loose meshwork of connective tissue containing only a few degenerating epithelial cells (fig. 2). In the 25 to 60-day specimens, the anterior lobe was structurally normal (fig. 3, 4) except for the presence of a small core of dense connective tissue which extended radially from the region of the hypophyseal cleft. Phagocytic cells containing large particles of cellular debris were scattered through this connective tissue.

In the 5-day specimens, no significant differences in the anterior lobe cells of grafts from injected and uninjected hosts could be detected. The cells in the peripheral area were normal in size, deeply stained[4] and showed comparatively little granular loss. The great majority were chromophiles. The 10-day specimens from uninjected

[3] Progynon-B, kindly supplied by the Schering Corporation through the courtesy of Dr. Erwin Schwenk.

[4] In all references to specific staining reactions, the description is of tissue stained by the Cleveland Wolfe technic (12).

hosts were similar. In the 20 to 60-day specimens from uninjected hosts, the cells were faintly stained, cells and nuclei were of normal size, there were few red nucleoli and the Golgi apparatus was not prominent (fig. 7). There were few chromophiles, both as compared with the number (in proportion to chromophobes) in normal pituitaries and in the 5 to 10-day specimens. Grafts from hosts injected 10 days or longer showed the structural features which are characteristic of the pituitary response to large amounts of estrogen (13, 14). The cells and nuclei were large, there

TABLE 1. SURVIVAL OF GRAFTS

Experimental period days	Injection begun	Hypophysectomy	No. of experiments	No. of grafts surviving	Total no. of experiments	% of grafts surviving	Shortest experiment in which graft did not survive
5	No injection	Total	1	1			
	2nd day	Total	1	1			
10	No injection	Total	2	2			
8, 10		Partial	2	2			
10	2nd day	Total	1	1			
5	2nd day	Partial	3	2	10	90.0	
25 to 60	No injection	Total	4	0			25 days
13		Partial	1	0	5	0.0	13 days
15 to 17	4th day	Total	3	2			17 days
25	2nd day	Total	1	0[1]			25 days
25	2nd day	Partial	1	0			25 days
30	17th day	Total	1	1			
30	21st day	Partial	1	0	7	42.8	30 days
18 to 30	No injection	Total	4	2			18 days
15 to 60		Partial	5	1	9	33.3	15 days
18 to 30	2nd day	Total	4	3			30 days
20 to 60	2nd day	Partial	5	4	9	77.7	30 days
16 to 20	4th to 9th day	Total	1	0			17 days
		Partial	3	3			
30	17th day	Partial	1	0			30 days
37	25th day	Partial	1	1	6	66.6	
Totals:	Male Donors		12	3		25.0	
13 to 60 days	Female Donors		24	14		58.3	
	Total		36	17		47.2	

[1] Connective tissue framework of anterior lobe more or less intact; nests of normal intermediate lobe cells extended about half-way around the periphery; no normal anterior lobe cells and no posterior lobe could be identified.

were many red nucleoli and the Golgi apparatus was hypertrophied. The majority of the cells were chromophobes, some with pale, fragmentary cytoplasm and indistinct cell membrane, others with deep blue cytoplasm and intact cell membrane (fig. 8). Many chromophobes contained small yellowish masses in the region of the Golgi apparatus. With increasing length of the injection period, the intensity of the reaction was increased, as indicated by a progressive increase in the number and size of the chromophobes and in the prominence of the negative image of the Golgi apparatus. Non-granular basophiles and a few granular basophiles were present in all speci-

mens; many of the latter showed evidence of granular loss. Eosinophiles were fairly numerous but less so than in normal pituitaries; granular loss was not marked. There was mild vacuolization of the cytoplasm of the type observed by Wolfe and Wright (14) in pituitaries of rats injected with estrogen for 30 days or longer. Extreme capillary distension similar to that described by Wolfe and Wright was not observed.

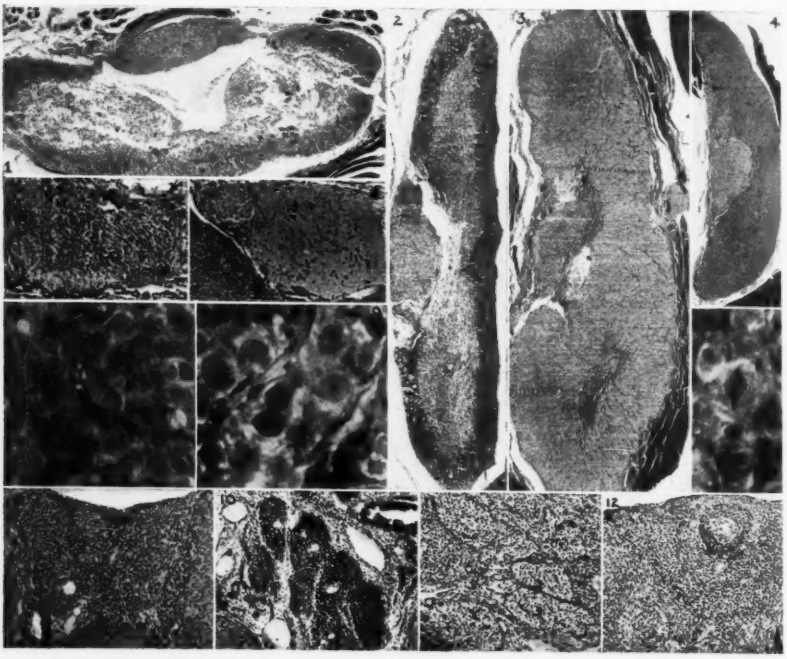


Fig. 1. Section of graft removed 5 days after implantation. ×25. Fig. 2. Section of graft removed 10 days after implantation. ×25. Fig. 3. Section of graft removed 30 days after implantation. Injected host. ×25. Fig. 4. Section of graft removed 30 days after implantation. Uninjected host. ×25. Fig. 5. Section showing structure of posterior lobe illustrated in figure 3. ×70. Fig. 6. Section showin structure of posterior lobe illustrated in figure 4. ×70. Fig. 7. Section of graft from uninjected host removed 30 days after implantation. ×560. Fig. 8. section of graft from injected host, removed 25 days after implantation. ×560. Fig. 9. Section showing interstitial tissue in ovary of normal rat. ×56. Fig. 10. Section showing interstitial tissue in ovary of hypophysectomized rat with non-surviving graft. ×56. Fig. 11, 12. Sections of ovaries showing other types of interstitial tissue reactions observed (see tables 3 and 4). ×56. Fig. 13. Section showing mitotic figure in graft removed 37 days after implantation. Host injected 13 days. ×560.

Mitotic figures were present in specimens from both injected and uninjected hosts (fig. 13).

Posterior lobe. In the 5-day specimens, the posterior lobe contained extensive areas of puple-stained, finely granular material. Also present were normal nuclei and large cells with cytoplasm unstained except for a few fine, pale yellow granules. The 10 to 20-day specimens were similar except that none of the purple-stained material was present. In the 25-day and in some 30-day specimens, the posterior lobe consisted chiefly of tissue which was entirely similar in appearance to the neuroglia of the normal posterior lobe (fig. 5). Scattered through this tissue were normal nuclei

and phagocytic cells loaded with debris. In two 30-day and two 60-day specimens,[5] the posterior lobe was packed with small cells which contrasted sharply with the larger anterior lobe cells (fig. 6). The significance of these cells could not be determined. There was no evidence that they penetrated the anterior lobe tissue; they extended for a short distance into the connective tissue core of the anterior lobe in one specimen. No posterior lobe could be identified in all 4 specimens.

Intermediate lobe. In the 5-day specimens, normal or degenerating intermediate lobe was present in the normal position. Normal intermediate lobe cells, arranged in nests and located above or at the side of the posterior lobe (fig. 3, 4, 5) were present in all except 2 of the 10 to 60-day specimens. In general, these cells were non-granular;

TABLE 2. FOLLICLES IN OVARIES OF HYPOPHYSECTOMIZED ANIMALS

Graft survived	Days uninjected	Days injected	Description of follicles
No (5 rats)	25 to 60	0	Few, majority atretic, none with antra
No (2 rats)	1 to 3	15 to 30	
½	21	0	
Yes[1]	16	14	
Yes[1] (2 rats)	3	12	Numerous, many normal with small antra,
Yes	0	18	atretic with fairly large antra, none mature[2]
Yes	10	0	As above but less numerous, atretic ma-
Yes (2 rats)	0	10; 25	ture follicles in animal with non-surviving
No	3	13	graft
Yes[1]	5	0	Atretic with large antra but none with an-
Yes[1]	0	5	tra normal. Atretic mature follicles in
Yes	30	0	5-day specimen from uninjected animal
Yes	0	30	

[1] Surviving grafts from male donors.

[2] A similar condition, associated with involuting corpora lutea was observed in 3 partially hypophysectomized animals injected for 13 to 14 days, one with non-surviving graft, 2 with surviving graft but less than one-fourth of their own pituitaries.

granular cells were present in a few specimens. Mitotic figures were observed in 4 specimens (injected hosts, male and female donors). Intermediate lobe tissue was most abundant in grafts from uninjected hosts and least abundant in female grafts from hypophysectomized, injected hosts. It was quite abundant in male grafts from hypophysectomized, injected hosts. In one such graft, intermediate lobe tissue survived although the anterior and posterior lobes did not (see footnote, table 1).

Functional activity of grafts. The hypophysectomized rats with non-surviving grafts showed the structural and weight changes which occur typically after hypophysectomy (tables 2–4), with two exceptions in which the findings were in some respects similar to those in rats with surviving grafts.

All of the hypophysectomized rats with surviving grafts showed a steady but very slight increase in body weight. This, together with the appearance and behavior of the animals, was sufficient to indicate that they were in good physical condition, but hardly sufficient to be considered conclusive

[5] From partially hypophysectomized, injected and uninjected and totally hypophysectomized, injected hosts.

evidence that the grafts were functional. Other findings relating to the functional activity of the grafts are presented in tables 2, 3 and 4. They are indicative of some, but on the whole comparatively little, activity.

The findings indicative of gonadotropic activity in the hypophysectomized rats with surviving grafts were of particular interest. There was evidence of mild follicle stimulation in the rats injected for 12 to 18 days (table 2) but no evidence that ovulation occurred or that corpora lutea were formed in either the injected or uninjected animals of this group.[6] The corpora

TABLE 3. FINDINGS IN UNINJECTED ANIMALS

Hypophysectomy	Graft survived	Exper. period days	Corpora lutea[1]	Interstitial tissue[2] Chromatin	Vaginal smears	Vaginal epithelium	Adrenal atrophy	Weight[3] Ovaries	Weight[3] Adrenals	Weight[3] Kidneys	Autopsy days after estrus
Total, operation during diestrus	No[4]	25 25 30 60	I-1	10 wheel cells	Cornified 4th day, mixed thereafter	Castrate	Marked	15.6 11.1— 5.0 6.9	17.2 16.4 14.3 7.4	679.7 741.4 712.1 671.8	
	Yes[4]	5	Large	S clumped		Stratified	Mild	12.5	22.4	—	2
Total, operation during estrus or diestrus	½ Yes No	21 30 30	I-2 I-1 None	10, 12 clumped 12 clumped 12 clumped	Leucocytes and epithelial cells throughout	Castrate	Marked	7.0 7.7 4.6	16.6 22.4 15.1	606.4 659.6 714.0	
	No Yes	18 10	No tissue			No tissue	No tissue	— 35.4	— 29.0	— —	
	Yes	10	Large	12 coarse		Low, muc.	Mild	26.8	26.3	763.9	
Partial, operation during estrus	Yes Yes	8 10	Large	9 normal 9, 12 coarse		Low, muc.	Mild None	20.1 40.8	28.4 37.5	864.6 804.0	
	No	15		11, 12 clumped		Stratified	Mild	20.5	20.3	618.2	
Partial, operation during diestrus	No[4] No No No	13 25 30 30	Normal Large Normal I-1	9 coarse 11 normal 9 coarse 12 normal	Irregular cycles; estrus 1 to 3, diestrus 3–15 days	Stratified Low, muc. Stratified Castrate	Mild Mild None None	19.0 23.3 25.3 14.7	33.1 30.5 44.1 41.8	1002.1 772.4 899.4 806.5	1 1 1 5
	Yes	60	Normal	9, 12 normal	13-day cycles	Diestrus	Mild	33.2	29.8	705.8	7

[1] *Normal*: comparable in size and appearance to those of normal cycle.
Large: intermediate between 'normal' and 'LARGE' (table 4) in size of corpora and cells; cells similar in appearance to those of LARGE corpora; subnormal number of corpora in some specimens.
I-1: involuting, structurally intact, mild or moderate degenerative changes.
I-2: in last stages of degeneration, few, small, beginning to show structural disintegration.

[2] Numerals refer to figures 9–12. S indicates some shrinkage but not as extreme as in fig. 10. Description of chromatin refers to changes indicative of hypophyseal deficiency in thecal nuclei (20). A few typical wheel cells with 'clumped' chromatin, none with 'coarse' chromatin.

[3] In mg. per 100 gm. body weight. Dash after ovary weight indicates presence of sufficient extra-ovarian tissue to account for approximately one-half of the recorded weight.

[4] Grafts from male donors.

present at the time of operation were maintained for 10 but not for 20 days in the uninjected rats (table 3); they underwent regression within 10 days in the injected rats (table 4). In this connection, it should be recalled that after hypophysectomy in the rat, the corpora lutea do not show marked degenerative changes until long after the operation (15). In nonhypophysectomized rats, the injection of large amounts of estrogen for approximately 10 days leads to the formation of corpora lutea comparable to those of late pregnancy (16). This is due to a modification of the corpora which form following ovulation; the number of these bodies is not increased. Estrogen

[6] Two rats came into estrus 2 to 3 days after hypophysectomy, but this could not be attributed to activity on the part of the grafts.

produces this effect upon the corpora lutea indirectly (17), presumably by stimulating the release of anterior pituitary luteinizing hormone. After long continued treatment of normal rats with estrogen, the corpora lutea disappear and the ovaries become atrophic (18). Greep (19) found that corpora lutea persisting after hypophysectomy in rats were not affected by estrogen or FSH but regressed within 10 days when LH was injected. The rapid regression of the corpora in the present experiments seems to indicate, therefore, that some luteinizing hormone was present in the injected animals

TABLE 4. FINDINGS IN INJECTED ANIMALS

Hypophysectomy	1st injection during	Graft survived	Days injected	Corpora lutea[1]	Interstitial tissue[2]		Days not injected	Vaginal smears after injection begun	Adrenal atrophy	Weight[3]		
					Chromatin		Estrus			Ovaries	Adrenals	Kidneys
Total	Estrus	No[4]	14	I-1	10	clumped	3 once	Cornified throughout	Mild	13.8—	18.9	—
		No	30	I-1	10	coarse	0		Marked	5.0	20.6	619.3
	Diestrus	No	13	I-1	S	clumped	3 none	Cornified after 1–3 injections	Mild	16.4	12.3	—
		No[4]	25	No tissue			0		Marked	18.4	17.7	856.1
		Yes[4]	5	Large	11	coarse	0		Mild	33.3—	33.3	850.7
	Estrus	Yes[5]	12	I-1	S, 10	coarse	3 once	Cornified throughout	Mild	25.0—	21.6	—
		Yes	18	I-2	9, S	normal	0		Mild	12.5	30.0	991.7
	Diestrus	Yes[4]	12	I-2	9, S	clumped	3 none	Cornified after 2–3 injections	Mild	10.7	20.0	—
		Yes	10	I-2	12	coarse	0		Mild	16.6	22.6	646.6
		Yes	25	I-2	S	coarse	0		Marked	12.5	26.9	795.4
		Yes[4]	14	I-2	S	clumped	16 none		Marked	10.0	13.1	—
		Yes	30	none	S	coarse	0		Marked	9.3	16.8	743.2
Partial	Estrus	Yes	12	LARGE	9	coarse	8 once	Cornified 4 days; mixed thereafter	None	37.5	59.6	—
		No[4]	10	Large	9, S	normal	20 thrice		None	16.2	32.2	748.1
		½	21	No tissue			0		No adr.	55.5	29.1	797.9
		Yes	25	Large	9, S	coarse	0		None	33.3	47.5	990.8
		No	30	I-1	12	coarse	0	Cornified 6 days; mixed 8 days; cornified thereafter	Mild	8.9	50.7	829.1
	Diestrus	No	5	Normal	9	normal	0	Cornified after 1–3 injections	Mild	21.2	23.6	758.8
		No	8	I-1	11	coarse	16 twice		Mild	23.5	29.4	—
		Yes[5]	13	I-1	S	clumped	3 none		Marked	10.5	10.5	—
		Yes[5]	14	I-2	S	coarse	3 none		Mild	11.3	11.3	—
		Yes	13	Large	9, 12	coarse	24 twice		Mild	14.6	18.0	668.6
		No	25	No tissue					None	5.1	43.2	1016.9
		Yes	30	None	12	coarse	0		None	13.7	47.5	979.9
		Yes	60	None	10	coarse	0		None	26.0—	44.1	826.6

[1] LARGE: comparable in size and appearance to those of late pregnancy. Others as in table 3.
[2] As in table 3.
[3] As in table 3.
[4] Grafts from male donors.
[5] Less than one-fourth of host's pituitary left.

for at least 10 days after the implantation of the grafts. It is possible that the luteinizing hormone, if present, was derived from the anterior lobe tissue which degenerated. However, the findings in the hypophysectomized rats with non-surviving grafts argue against this possibility. Thus, the results of these experiments do not necessarily indicate that the physiologic response of the grafts to estrogen was qualitatively abnormal.

The length of time during which normal corpora lutea were maintained in the ovaries of the partially hypophysectomized, injected rats was roughly proportional to the amount of pituitary tissue present and did not exceed 25 days in any case. Normal corpora are maintained, as a rule, for at least 30 days in normal mature rats similarly injected (author's unpublished observa-

tions and Wolfe and Wright, 14). The results of the present experiment suggest, therefore, that ovarian atrophy resulting from the injection of estrogen takes place more rapidly in animals with hypophyseal deficiency than in normal animals.

The significance of the association of a marked structural response of the grafts to estrogen with evidence of very little functional activity is not clear. The degree of functional activity may well have been dependent upon the technical procedure but it is quite possible that it was dependent upon other factors. It should be noted that, although the grafts from the injected hosts showed the histologic features which are typical of the pituitary response to estrogen, these findings do not prove conclusively that the structural response of the grafts to estrogen was normal in every respect. Degranulation of the chromophiles is an essential feature of the normal response (13). It cannot be said with certainty that this effect was produced in the present experiments, since, in so far as could be determined without cell counts, there was no significant difference in the proportion of chromophiles in grafts from injected and uninjected hosts, respectively. The results do indicate, however, that some, if not all, of the effects of estrogen upon the structure of the anterior lobe can be produced after the nervous connections of the pituitary have been severed.

The marked difference in the survival of grafts in injected and uninjected hosts is indicative of a stimulative action of estrogen upon the pituitary. Such an influence is further indicated by the marked difference in the size of the grafts from injected and uninjected hosts (compare fig. 3, 4). This difference appeared to be due in part to more extensive regeneration in the injected animals and not altogether to the difference in the size of the cellular elements. The stimulative effect of estrogen indicated by these findings may have been produced by direct action or it may have been due indirectly to the influence of estrogen upon the blood supply. It is quite possible that both of these factors were involved. The favorable influence upon regeneration indicates that estrogen is able to stimulate mitotic activity in the anterior lobe after the nervous connections of the gland have been severed.

SUMMARY

The survival, structure and function of pituitary grafts in untreated female rats and in female rats injected with estrogen was investigated. Estrogen was found to exert a favorable influence upon the survival of the grafts. Grafts from female donors survived better than did those from male donors, regardless of the treatment of the hosts. All three lobes could be identified in surviving grafts. The anterior lobe was observed to undergo partial degeneration followed by regeneration; these changes were not dependent upon the presence of injected estrogen; however, regeneration was more extensive in the injected rats. Grafts which had undergone regeneration were structurally normal. Those from injected hosts showed the histologic features which are characteristic of the pituitary response to estrogen; those from uninjected hosts showed a relatively small proportion of

chromophiles. With respect to the functioning of the grafts, the findings were indicative of some, but comparatively little, activity. The significance of these findings has been discussed.

The results of the experiments indicate that some, if not all, of the effects of estrogen upon the structure of the anterior lobe can be produced in the absence of the nervous connections of the pituitary. They indicate further that the anterior lobe is capable of regenerating after having undergone partial degeneration. The observations on the effect of estrogen upon the survival and regeneration of the grafts afford conclusive evidence that this hormone stimulates the pituitary.

REFERENCES

1. Schweizer, M., H. A. Charipper and H. O. Haterius: *Endocrinology* 21: 30. 1937.
2. Greep, R. O.: *Proc. Soc. Exper. Biol. & Med.* 34: 754. 1936.
3. May, R. M.: *Compt. rend. Soc. de Biol.* 124: 920. 1937.
4. Martins, T.: *Compt. rend. Soc. de Biol.* 123: 702. 1936.
5. Desclin, L., and Ch. Grégoire.: *Compt. rend. Soc. de Biol.* 121: 1366. 1936.
6. Hohlweg, W., and K. Junkmann: *Klin. Wnschr.* 11: 321. 1932.
7. Hill, R. T., and W. U. Gardner: *Proc. Soc. Exper. Biol. & Med.* 34: 78. 1936.
8. Buxton, C. L.: *Anat. Rec.* 64: 277. 1936.
9. Emery, F. E.: *Anat. Rec.* 66: 253. 1936.
10. Richter, C. P., and J. F. Eckert: *Endocrinology* 21: 481. 1937.
11. Kylin, E., and A. Koranyi: *Klin Wnschr.* 17: 668. 1938.
12. Cleveland, R., and J. M. Wolfe: *Anat. Rec.* 51: 409. 1932.
13. Wolfe, J. M.: *Proc. Soc. Exper. Biol. & Med.* 32: 1192. 1935.
14. Wolfe, J. M., and A. W. Wright: *Endocrinology* 23: 200. 1938.
15. Smith, P. E.: *Am. J. Anat.* 45: 205. 1930.
16. Wolfe, J. M.: *Proc. Soc. Exper. Biol. & Med.* 32: 757. 1935.
17. Ellison, E. T., and J. C. Burch: *Endocrinology* 20: 746. 1936.
18. Diaz, J. T., D. Phelps, E. T. Ellison and J. C. Burch: *Am. J. Physiol.* 121: 794. 1938.
19. Greep, R. O.: *Endocrinology* 23: 154. 1938.
20. Selye, H., J. B. Collip and D. L. Thomson: *Endocrinology* 17: 494. 1933.

ENDOCRINE FUNCTION AND AMYLASE ACTIVITY

II. CHANGES IN ACTIVITY OF BLOOD SERUM AMYLASE IN RESPONSE TO CHANGES IN ADRENAL CORTICAL FUNCTION IN THE DOG AND RABBIT

OLIVER COPE, ISRAEL KAPNICK, ADRIAN LAMBERT, T. DENNIE PRATT AND MAX G. VERLOT[1]

From the Surgical Laboratories of the Harvard Medical School at the Massachusetts General Hospital

BOSTON, MASSACHUSETTS

THE PRIMARY function of the adrenal cortex has not as yet been solved. At the moment there are three major theories concerning its action. The first suggests that the adrenal cortex exerts its influence through control of carbohydrate metabolism. This point of view has been championed by Britton and Silvette (1), and more recently by the experiments of Long and Lukens (2). The second theory has evolved from experiments by Loeb (3), and Harrop (4), and their associates. Changes in inorganic salt metabolism result following the removal of the adrenal cortex in animals, and in patients suffering from Addison's disease. Swingle and his co-workers (5) are responsible for the third theory, that the adrenal cortex controls exchange of fluid within the body.

Other theories of the action of this gland have been suggested. No attempt is made here to cover what has become a vast literature. It is obvious that further evidence will have to be obtained before these different points of view can be reconciled.

Since a change in blood serum amylase activity occurs following the removal of the anterior pituitary in the dog (6) it was decided to study the adrenalectomized animal in the hope that this might throw further light on the relation of the adrenal cortex to carbohydrate metabolism. Experiments carried out on adrenalectomized dogs and rabbits, together with results following injection of adrenal cortical extract and desoxycorticosterone acetate, are reported in this paper.

METHODS

Both the dog and rabbit were used in these experiments. Serum amylase activity was measured by the same method as that used in the previous work (6). The figures and charts are based upon the amount of alcohol-insoluble glycogen lost during a 3-hour period of digestion. In dog serum the activities of 2.5% and 5% concentrations were measured. Because the

[1] Graduate Fellow of the Belgian American Educational Foundation, Inc.

activity of amylase in blood serum of the normal rabbit is less than in that of the dog, 5% and 10% serum concentrations were used. The pH, substrate and temperature conditions were the same for both animals.

The non-protein nitrogen, serum protein, blood sugar and chloride were measured by the accepted laboratory procedures. Serum sodium and potassium were estimated by the methods of Butler and Tuthill (7) and Fiske and Litarczek (8) respectively. The hematocrit was determined in the majority of readings by using isotonic oxalate solution, volume correction being made in the calculation.

In the dog, adrenalectomy was accomplished by removing one adrenal at a time through a lumbar incision, sometimes resecting the twelfth rib. In the rabbit, one or both adrenals were removed at a time through an abdominal incision. Careful search was made for accessory cortical tissue. Ether anesthesia was used. Adrenal cortical extract, eschatin,[2] was administered, in amounts quoted in the individual experiments, following the removal of the second adrenal in order to exclude as far as possible the effects of operation and anesthesia. The development of typical adrenal cortical insufficiency and careful postmortem gross observation assured the complete removal of all adrenal cortical tissue.

EXPERIMENTS

The experiments were divided into 4 parts. Ten dogs and 7 rabbits in all were used.

Adrenal cortical insufficiency in the dog. Five dogs (no. 1–5, 1 female and 4 males) were adrenalectomized. A maintenance dose of cortical extract was given subcutaneously in the first days after the second operation. The animals were then observed as they slipped into terminal cortical insufficiency. The blood serum amylase activity, both of 5% and 2.5% serum, was observed in the normal and insufficient states. In order to judge objectively the degree of cortical insufficiency, the blood N.P.N., sugar, hematocrit, serum protein, sodium, potassium and chloride were measured. The data for the first 4 animals are given in table 1. The data on *dog 5* are charted in figure 1.

After removal of the second adrenal gland in the first 4 animals, in spite of the administration of cortical extract, there was a precipitous rise in serum amylase activity to a level above the normal range. The level reached either before or immediately after the omission of cortical extract was more than twice the normal (*dog 3, 4*). The activity of 2.5% serum was greater than that of 5% serum before operation. During the administration of extract the animals were clinically normal. They were active, eating well, and they showed no loss of weight or hair. Following withdrawal of the extract the animals progressed into typical cortical insufficiency and the expected changes in the other blood findings, recorded by other workers, occurred. No further rise in serum amylase activity took place, however.

In the fifth animal (fig. 1) following removal of the second adrenal gland more than double the so-called maintenance dose of extract was administered.

[2] We are indebted to Parke, Davis & Co. for a generous supply of this cortical extract.

The animal remained in excellent condition. There was no change from the normal in the N.P.N., hematocrit, serum protein, sodium, potassium or chloride, yet the rise in serum amylase activity occurred. The activity of

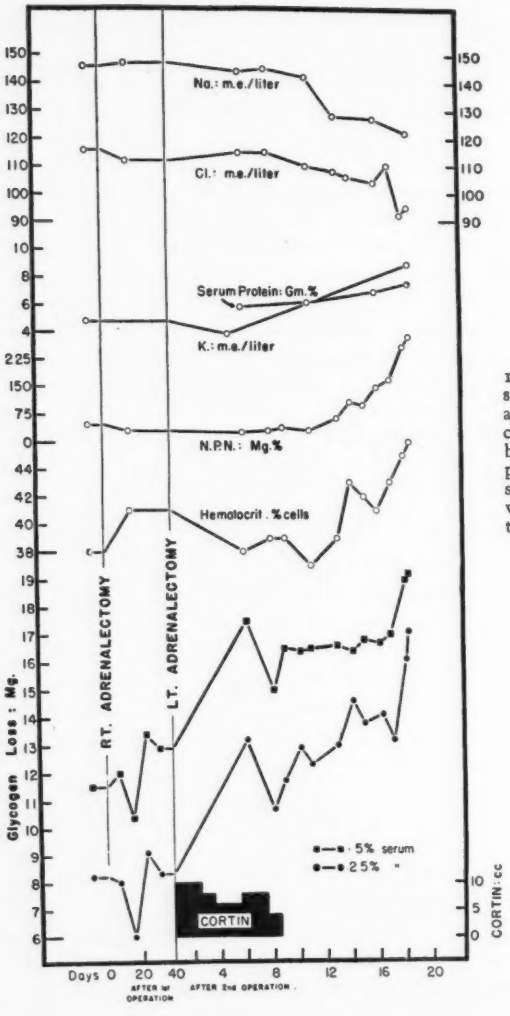


Fig. 1. *Dog 5*, male, weight 15.0 kg. ADRENAL CORTICAL INSUFFICIENCY. Blood serum amylase activity, 5 and 2.5% serum concentrations, with corresponding blood N.P.N., hematocrit, serum protein, Na, K, and Cl. Blood sugar on 17th day postoperative was 56 mg. %. The cortical extract used was eschatin.

2.5% serum after operation, with the dog on cortical extract, was equal to that of 5% before operation. Following withdrawal of cortical extract there was a further rise in serum amylase activity, and as the clinical signs of insufficiency developed, the expected changes in the other blood constituents occurred. The blood serum sodium and chloride fell, the potassium rose. The serum protein and hematocrit showed hemoconcentration with loss of fluid

from the serum. The change in serum amylase activity, however, significantly preceded the other observed changes by more than 24 hours. This precedence is visible in the 2.5% serum curve because in this range of activity the lower

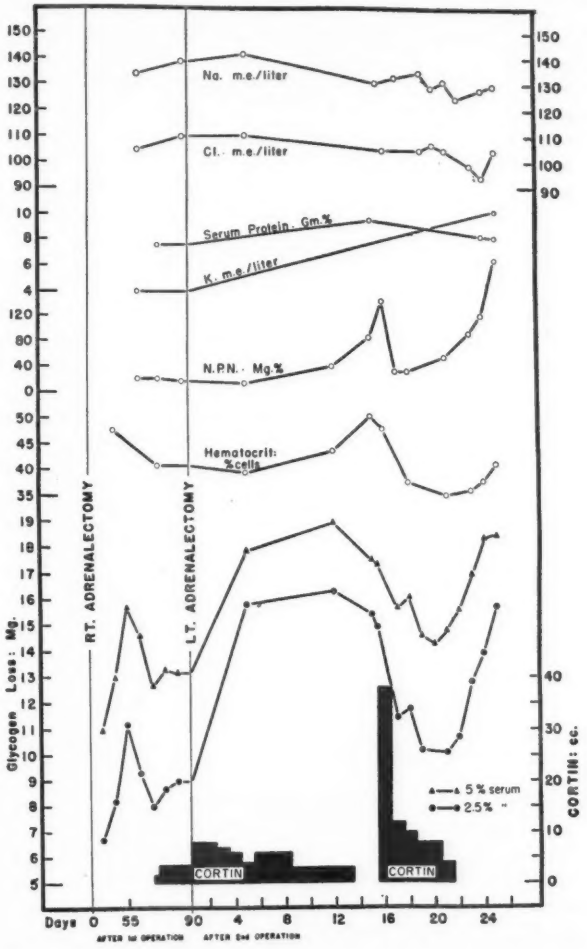


Fig. 2. *Dog 6*, male, weight 9.5 kg. ADRENAL CORTICAL INSUFFICIENCY, CORTICAL EXTRACT., Blood serum amylase activity, 5 and 2.5% serum concentrations with corresponding blood N.P.N. hematocrit, serum protein, Na, K, and Cl. Blood sugars taken in severe adrenal insufficiency, 15th day postoperative, 45 mg. % and 24th day postoperative, 92 mg. %. The cortical extract used was eschatin.

serum concentration is the more sensitive. When terminal insufficiency was reached the animal was killed by bleeding.

Since operation and ether anesthesia might have contributed to the rise in serum amylase activity seen after adrenalectomy, control operations were performed. Dogs subjected to stomach operations with ether anesthesia

TABLE I

Dog	Date	Adrenalectomy	Wt.	Cortin	AMYLASE Serum 5%	AMYLASE Conc. 2.5%	Blood Sugar	N.P.N	Hem.	Na	Cl	Remarks
			kg.	cc.			mg. %	mg. %	% cells	m.eq.	m.eq.	
1 ♀	11/24/37				11.1	6.8						
	11/29				12.6	9.0						
	2/ 1/38	Rt.										
	2/23		8.5									
	2/28	Lt.		1.0								
	2/29 to 3/ 3			0.5/day								
	3/ 4			1.0								
	3/13				17.7	14.4						
					17.4	14.7						
	3/14						65	134	42	127		
	3/15							200				Died
2 ♂	3/ 1/38				13.6	8.8						
	3/ 8				11.7	7.1						
	3/31	Rt.										
	4/20		25.9		13.4	8.4						
	4/21		26.1		13.6	8.3	74	30				
		Lt.										
				7.0	13.3	9.4						
	4/22 to 4/24			1.8 av.								
	4/25						85	46	29			
	4/26						72					
	4/27				18.5	14.6			31			
	4/30				18.2	14.7						Died
3 ♂	5/24/38				13.7	8.7						
	5/25				12.7	7.7						
	5/26				12.5	7.4						
		Rt.										
	8/25				15.4	10.7						
	9/15	Lt.		2.8								
	9/16 to 9/18			4.0 av.								
	9/19		17.0	2.0								
	9/20		16.6	2.0	16.9	13.8						
	9/21 to 9/23			1.0/day								
	9/24				17.1	13.9	85					
	9/26											Died
4 ♂	9/14/38		10.3		14.1	10.0						
					14.5	9.0						
		Rt.										
	9/29		9.7		15.3	12.4						
	10/ 5 to 10/ 9	Lt.		4.0 av.								
	10/10 to 10/13			2.0 av.								
	10/14			5.0								
	10/15				16.5	15.7	83	32	30	139		
	10/16				16.9	15.9						
	10/17				16.7	15.3						
					16.6	15.8				134		
					16.4	15.3		214		132	103	Killed

lasting as long as 4 hours were studied. No significant change from the normal preoperative blood serum amylase activity was observed during or after operation.

Effect of cortical extract and desoxycorticosterone acetate on the adrenalectomized dog. It became obvious from the first 5 experiments quoted in the previous section that if the blood serum amylase activity of the adrenalectomized dog was to be controlled within normal limits by injection of cortical extract, it could only be done with doses larger than those required for so-called maintenance. The following experiments were, therefore, performed on 3 male dogs, no. 6, 7, 8. The data are recorded in figures 2 and 3 and table 2.

TABLE 2

Dog	Date	Cortin	AMYLASE		Blood Sugar	N.P.N	Hem.	Serum Prot.	Na	K	Cl	Remarks
			Serum	Conc.								
		cc.	5%	2.5%	mg. %	mg. %	% cells	gm %	m.eq.	m.eq.	m.eq.	
7 ♂	1/24/39[1]		9.6	5.7		40	49	6.3	143		106	
	Rt. ad.[2]											
	1/26		14.2	9.5								
	1/28		13.7	9.0								
	1/31		13.0	9.1								
	2/9[3]		10.6	6.2	55	38					112	
	2/11	14	9.5	6.3		73		7.0	143	7.1		
	2/12	21										
	2/13		10.5	5.9								
	Lt. ad.[4]	40										
	2/14	20	11.9	7.7								
	2/15	21	15.0	10.3								
	2/16	15	15.9	11.7								
	2/17 to	15/	16.5	11.2		45	43		145		109	Abscess
	2/20	day	16.8	11.6		52		6.7		4.5		drained
	2/21		17.5	14.0								
	2/22				42	85		6.6	141		108	
	2/23		16.8	12.5		44	49					
	2/24		16.4	12.3	65	64	50					
	2/25		17.5	13.7								
	2/26		17.1	13.6	85	112	52			5.9		
			17.8	14.4	78	121	52	6.0	131		106	Killed

[1] Weight was 10.0 kg.
[2] Right adrenalectomy.
[3] Weight was 9.8 kg.
[4] Left adrenalectomy.

In *dog 6* (fig. 2) bilateral adrenalectomy was done with 90 days between stages. Because a significant slight rise in serum amylase activity was observed after the first adrenalectomy, injection of cortical extract was started 14 days before removal of the second adrenal. (The amylase activity had already fallen to normal before the injection of the extract.) Injection of extract was continued through the 12th day after the second operation. The dose injected corresponded for the weight of the dog to that given *dog 5*. (This dose has been insufficient to prevent a nearly maximal rise in amylase activity in *dog 5*, fig. 1). An abrupt rise in serum amylase activity occurred. The activity of 2.5% serum became considerably higher than that of 5% serum before operation. No change in the other determinations occurred. The amount of extract was, therefore, adequate for so-called maintenance.

On the 14th day after removal of the second adrenal, cortical extract was omitted and the dog allowed to become insufficient. In 3 days the animal was prostrate. No further elevation of the serum amylase activity occurred but

the changes typical of severe cortical insufficiency occurred in the other blood findings. When the animal was so weak it could not stand, intravenous and subcutaneous eschatin, totalling 38 cc. in the first 24 hours, was given. Intravenous saline and glucose were also administered. Twelve and 10 cc.

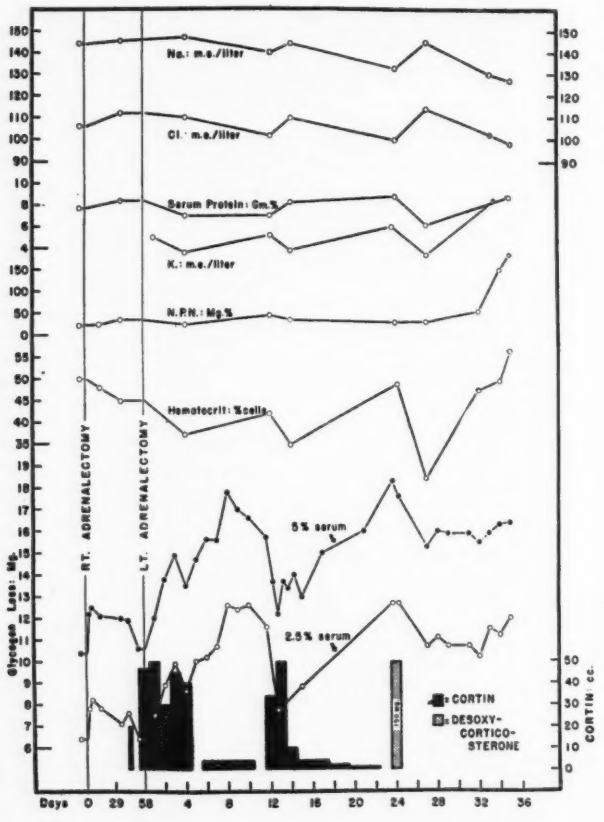


Fig. 3. *Dog* 8, male, weight 12.0 kg. ADRENAL CORTICAL INSUFFICIENCY, CORTICAL EXTRACT, DESOXYCORTICOSTERONE ACETATE. Blood serum amylase activity, 5 and 2.5% serum concentrations with corresponding blood N.P.N., hematocrit, serum protein, Na, K, and Cl. The cortical extract used was eschatin. The desoxycorticosterone acetate was given in sesame oil subcutaneously.

of cortical extract were given in the following 2 days respectively. Clinically the animal recovered promptly. The blood nitrogen, electrolytes and hematocrit findings returned to normal. The amylase activity of the serum also returned to just within the upper limit of the normal range for the first time since removal of the second adrenal. It was thus proved that if relatively large or truly adequate doses of cortical extract were administered the amylase activity could be brought within normal limits. After 6 days on extract the experiment was terminated by allowing the animal to go into insufficiency

for the second time. The changes in the blood occurred rapidly. The rise in serum amylase activity preceded significantly the other changes. In 4 days the animal was prostrate and was killed.

In *dog 7* (table 2) bilateral adrenalectomy was performed with 20 days between stages. After removal of the first gland a significant rise in serum amylase activity was observed which disappeared spontaneously in 2 weeks. Administration of large doses of cortical extract was started 2 days before removal of the second adrenal and continued through the seventh postoperative day. In spite of the huge dose of extract the amylase activity had risen by the 2nd postoperative day and remained elevated. On the 4th postoperative day a subcutaneous abscess of the second wound was recognized and evacuated. It was felt that this infection might well have influenced the response to cortical extract and, therefore, the extract was continued. The wound inflammation subsided very promptly. The clinical condition of the animal remained excellent. There was, however, no drop in the amylase activity. The other blood findings were normal.

Because of the complicating factor of mild sepsis, extract was discontinued. A further rise in the amylase activity to a maximal level promptly occurred. Cortical insufficiency and the typical nitrogen, electrolyte and hematocrit findings slowly developed. The animal was killed in a moderate degree of insufficiency on the 6th day after extract withdrawal.

In *dog 8* (fig. 3) bilateral adrenalectomy was performed with a 58-day interval between stages. No rise to above normal limits was observed in the serum amylase activity after removal of the first gland. Huge doses, larger in actual amounts and by weight than those given *dog 7*, were administered, starting before operation and continuing through the 4th postoperative day. In spite of these doses the amylase activity increased moderately. For the next 6 days the amount of extract was cut to 4 cc., which was still more than a maintenance dose for an animal of this size. The amylase activity rose to nearly a maximal level. On the 12th and 13th days, large doses of extract were again administered. There was a prompt drop in serum amylase activity to within normal levels.

The animal was again placed on a maintenance dose through the 22nd postoperative day. The serum amylase activity slowly climbed to the same high level previously reached on the maintenance dose. Throughout this entire postoperative period the animal's clinical condition was excellent. With the exception of the blood taken on the 24th postoperative day, no abnormality of the other blood findings was demonstrated. On the 24th day, when the amylase activity was at its height, the blood sodium was 133 m.eq., the chloride at the lower limit of normal. The hematocrit and serum protein suggested slight hemoconcentration but the N.P.N. was 30 mg.

After the blood determinations on the 24th day, 150 mg. of desoxycorticosterone acetate in oil[3] was given. The entire dose was given subcutaneously at one time; in order to increase the area of absorption, only a few cubic centi-

[3] Desoxycorticosterone acetate, 5 mg. in 1 cc. sesame oil, was made available to us through the interest and generosity of Dr. S. J. Thannhauser, and the Ciba Company.

meters were injected at any one site. In the next 4 days the amylase activity dropped to just above the upper limit of normal activity. There was a simultaneous rise in serum sodium and chloride, and a rehydration of the blood. The N.P.N. remained within normal limits.

No further desoxycorticosterone or cortical extract was given. The animal was allowed to go into terminal insufficiency. From the 28th to 32nd day the amylase activity remained constant at a moderately elevated level. In the last 3 days there was a rise to a slightly higher but not maximal level. The final blood was taken when the animal was prostrate. It is interesting that this is the single exception when the serum amylase was not at a maximum of activity in terminal insufficiency.

In the last 3 days hemoconcentration occurred; the serum sodium and chloride fell and the N.P.N. rose as expected.

In this experiment it has been shown that the serum amylase activity of the adrenalectomized dog can be brought within normal limits if adequate amounts of cortical extract are given. A reduction in activity can likewise be accomplished with large doses of desoxycorticosterone acetate.

Effect of desoxycorticosterone acetate in the normal dog. Two normal female dogs were injected subcutaneously with desoxycorticosterone acetate in oil. In the first dog, no. 9, weight 8.25 kg., 10 mg. per day was injected for 17 days. There was a slight but significant slow fall in serum amylase activity from an average control figure of 10.2 mg. (5% serum) to 7.6 mg. on the 12th day of injection. Thereafter there was a return rise to 8.5 mg. on the last day of injection when the animal was killed. No change from the normal was observed in blood N.P.N. sugar, protein, hematocrit, serum sodium, potassium or chloride. In the second dog, no. 10, weight 10 kg., 20 mg. per day was injected for 11 days. A slight but not significant drop in serum amylase activity occurred.

Adrenal cortical insufficiency in the rabbit. Adrenal cortical insufficiency is difficult to reproduce with regularity in the rabbit. There are two reasons for this difficulty. First, in order to excise the right gland in entirety it is necessary to remove a portion of the vena cava. Operative shock is increased if too much of the vena cava is removed. Second, the existence of accessory cortical tissue is common in the rabbit. These embryological remnants are difficult or impossible to find at the time of operation and are not visible until hypertrophied after a period of relative adrenal insufficiency. Because of them, about 20% of rabbits survive bilateral adrenalectomy indefinitely, if well cared for during the immediate postoperative period.

In spite of these drawbacks the rabbit was chosen for study because of already known differences in behavior in carbohydrate metabolism between it and the dog. Only those animals surviving a sufficiently long time after operation to exclude operation and anesthesia as complications have been included.

In figure 4 are shown the serum amylase findings in 3 rabbits surviving adrenalectomy from 10 to 15 days. All died in typical insufficiency with progressive muscular weakness and collapse. The serum amylase activity fell significantly in 2, remained unchanged in one. In figure 5 the data of 4 rabbits

surviving adrenalectomy for long periods are shown. In the first 2 weeks, signs of moderate insufficiency appeared; the animals were weak and lost weight. The course of the serum amylase activity after operation was unchanged in one, descended in one, fluctuated in 2 in the first week, with a slightly lower than control activity in the second week.

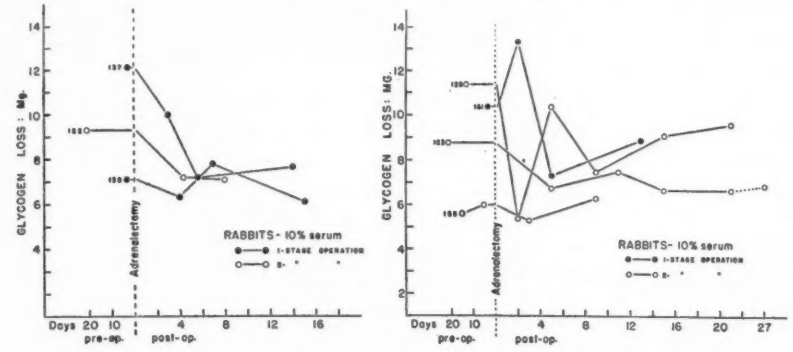


Fig. 4 (left). ADRENALECTOMIZED RABBITS. Blood serum amylase activity, 10% serum concentration *Rabbit 122* died 10 days, no. *137*, 14 days ,and no. *158*, 15 days after adrenalectomy. ●—● indicate animals adrenalectomized in 1 stage, ○—○ animals adrenalectomized in 2 stages. Fig. 5 (right). ADRENALECTOMIZED RABBITS. Blood serum amylase activity, 10% serum concentration. *Rabbit 123* died 82 days, no. *128*, 133 days after adrenalectomy. No. *155* received 4 cc. of cortical extract at operation. No. *155* was killed 83 days, no. *161*, 93 days after adrenalectomy

DISCUSSION

It is obvious from the experiments quoted in this paper that the adrenal cortex exerts a control over the blood serum amylase activity in the dog. With diminished function of the adrenal cortex there is a rise in amylase activity. The range of the amylase activity from normal levels to the maximum encountered in cortical insufficiency is wide. The maximum level is higher than that found under other circumstances, such as acute pancreatitis in the human being. It is significantly higher than the level of activity found following hypophysectomy in the dog (6).

The change in amylase activity in experimental adrenal insufficiency is the most sensitive index of diminished cortical function yet observed in the blood of the dog. A nearly maximal level of serum amylase activity is observed in mild cortical insufficiency at times when no other blood finding is abnormal and the dog is free of clinical signs of insufficiency. Larger doses of cortical extract are required to control the serum amylase activity within normal limits than to maintain the electrolyte and blood nitrogen figures in the normal range. It can, therefore, no longer be said that an adrenalectomized dog, kept alive on cortical extract, is receiving sufficient hormone for complete maintenance if it is merely clinically well and its blood N.P.N. and serum sodium are normal.

In 4 of 7 of the adrenalectomized dogs in which the serum amylase activity was observed between removal of the first and second adrenal

gland, there was a significant slight rise in activity which returned to normal spontaneously before the second operation. It has long been known that following unilateral adrenalectomy in experimental animals there occurs a so-called compensatory hyperplasia of the cortex of the remaining gland. Up until the present time no objective blood determination has been found sensitive enough to measure the degree of insufficiency supposedly necessary to call forth this compensatory hyperplasia. It is suggested that the rise in serum amylase activity seen in these 4 dogs may have represented a sign of such an insufficiency.

Another indication of the sensitivity of serum amylase activity as a measure of cortical insufficiency in the dog is the effect of operation and anesthesia in increasing the requirement of cortical extract. This is particularly evident in *dog 8* (fig. 3). Doses of extract which were able to bring the amylase activity within normal limits 12 days after operation were unable to control the amylase in the first 3 days after operation. This is in keeping with what is already known concerning the relation of the adrenal cortex to shock.

The rise in serum amylase activity in the cortically insufficient dog is the most dramatic evidence so far presented of a rôle played by the adrenal cortex in carbohydrate metabolism. Although hypoglycemia is observed sometimes in cortical insufficiency, in our experience it is by no means always present. In the experiments reported here, for example, in only one animal was a blood sugar as low as 45 mg. observed in advanced insufficiency (fig. 2, legend).

It is important that desoxycorticosterone acetate, as well as adrenal cortical extract, should have the property of decreasing the serum amylase activity of the insufficient animal. Desoxycorticosterone acetate, a single chemical entity, has been shown by others to correct the electrolyte abnormalities and be sufficient for life maintenance. That it influences a carbohydrate enzyme system as well, points to a common origin, as yet unsolved, for the two effects. Had cortical extract alone possessed this double property, it might have been assumed that two or more chemical substances were present in the extract.

No stress is laid on the drop in serum amylase activity observed in the normal dog injected with desoxycorticosterone. As with insulin, reported in a later paper (9), injection of an active substance into an intact animal calls forth a more complicated response than that seen in the ablation experiment of adrenal insufficiency.

The significance of the reported change in serum amylase activity is not clear. The origin or function of amylase in blood serum is not as yet known. That it may in some way be connected with liver amylase function is suggested by the fact that liver amylase activity is also increased in adrenal insufficiency. This work forms the basis of a subsequent paper (10). It is not concluded that the primary effect of the adrenal cortical hormone is to control either amylase activity or carbohydrate metabolism. That a relationship to both exists is obvious.

None of the electrolyte or nitrogenous changes known to occur in experimental adrenal insufficiency in dogs could be responsible for the observed amylase changes. Sodium and potassium ions in the concentrations occurring in the blood serum, for example, do not exert a significant influence on the amylase enzyme system. The hemoconcentration which also occurs is in itself insufficient to account for an increase in amylase concentration necessary to produce the observed changes in activity. Besides, it should be emphasized that the rise in serum amylase activity occurs in the absence of demonstrable changes in hemoconcentration and electrolyte concentration.

Other evidence against the control of serum amylase activity as a primary function of the adrenal cortex is the observation that the rise seen in the insufficient dog is absent in the rabbit. What effect is seen in the adrenalectomized rabbit is in the reverse direction.

SUMMARY

In adrenal cortical insufficiency in the dog there is a rise in blood serum amylase activity. The level of activity reached in advanced insufficiency is higher than that observed in anterior pituitary insufficiency.

The change in serum amylase activity is the most sensitive objective measurement in the blood of cortically insufficient dogs yet determined. In a dog developing insufficiency a significantly high level of activity is reached before any changes in nitrogenous or electrolyte substances of the blood are observed.

Both adrenal cortical extract and desoxycorticosterone acetate depress the elevated serum amylase activity of the insufficient dog.

The relation of adrenal insufficiency to serum amylase activity in the dog represents the most dramatic evidence so far observed of a part played by the adrenal cortex in carbohydrate metabolism.

In adrenalectomized rabbits no rise in serum amylase activity is observed. In half the animals a significant fall took place.

It is not concluded that the primary function of the adrenal cortex is control of the amylase system or of carbohydrate metabolism. The amylase changes observed, as well as those of electrolytes reported by others, are presumably secondary to some unsolved primary function of the gland.

REFERENCES

1. Britton, S. W., and H. Silvette: *Am. J. Physiol.* 118: 594. 1937.
2. Long, C. N. H., and F. D. W. Lukens: *J. Exper. Med.* 63: 465. 1936.
3. Loeb, R. F., D. W. Atchley, E. M. Benedict and J. Leland: *J. Exper. Med.* 57: 775. 1933.
4. Harrop, G. A., L. J. Soffer, R. Ellsworth and J. H. Trescher: *J. Exper. Med.* 58: 17. 1933.
5. Swingle, W. W., W. M. Parkins and A. R. Taylor: *Am. J. Physiol.* 116: 430. 1936.
6. Cope, O., A. Hagströmer and H. Blatt: *Am. J. Physiol.* 122: 428. 1938.
7. Butler, A. M., and E. Tuthill: *J. Biol. Chem.* 93: 171. 1931.
8. Fiske, C. H., and G. Litarczek: in Folin, O.: Lab. Manual Biol. Chem., 5th ed., 1934.
9. Cope, O., I. Kapnick, A. Lambert, T. D. Pratt and M. G. Verlot: *Endocrinology* 25: 248. 1939.
10. In preparation.

ENDOCRINE FUNCTION AND AMYLASE ACTIVITY

III. FURTHER OBSERVATIONS OF BLOOD SERUM AMYLASE ACTIVITY IN RELATION TO PITUITARY, PANCREAS AND THYROID FUNCTION IN THE DOG AND RABBIT

OLIVER COPE, ISRAEL KAPNICK, ADRIAN LAMBERT, T. DENNIE PRATT AND MAX G. VERLOT[1]

From the Surgical Laboratories of the Harvard Medical School at the Massachusetts General Hospital

BOSTON, MASSACHUSETTS

THE PREVAILING concept among clinicians regarding the enzyme amylase in the blood serum is that the enzyme is a functionless product of the acinar cells of the pancreas.[2] This concept is based on the earlier physiological work which considered changes in serum amylase activity as resulting from alterations in the external secretory function of the pancreas. Thus the abrupt transient rise in serum amylase activity seen in acute pancreatitis in the human being or following pancreatic duct ligation in the dog, has been considered to result from the backing up in the blood of unexcreted amylase intended for the duodenum.

The early attempts to discover an endocrine relation to amylase function in blood were confusing. For example, changes in function were reported in human diabetes mellitus by some observers only. Pancreatectomy in the dog was reported to be followed by a rise in serum amylase activity (3); later it was shown that a drop in activity occurs (4). Not until 1933 (5) was agreement reached that following insulin withdrawal in the pancreatectomized dog a drop in serum amylase activity does occur. It is probable that incomplete operative removal of the pancreas and inadequate chemical methods of amylase measurement were responsible for the confusion.

Receiving encouragement from the work of Scharles, Robb and Salter (6), and Hayes and Salter (7), on changes in rat liver amylase activity in response to thyroid hormone and hypophysectomy, the current opinion that the endocrine glands have little or nothing to do with serum amylase function, has been challenged by work from this laboratory. An abrupt sustained rise in amylase activity was observed following hypophysectomy in the serum of the dog (8). In the previous paper (9) a similar rise to an even higher level of activity has been reported in the adrenalectomized dog, whereas a slight fall or no change in activity was observed following removal of the adrenals in the rabbit. It is, therefore, obvious that the endocrine

[1] Graduate Fellow of the Belgian American Educational Foundation, Inc.

[2] Recent reviews of the literature are available in the papers of Elman (1) and Friedman and Thompson (2).

system does exert a control over the amylase system in the blood. The present experiments were undertaken to extend the knowledge of this endocrine influence.

METHODS

Both the dog and rabbit were used in these experiments. Serum amylase activity was measured in part by the method used in the previous papers (8, 9) and in part by a rapid nephelometric method to be described elsewhere. The values by the rapid method are expressed in the same terms as the slow method, that is, glycogen loss. The figures and charts read as in the previous papers (8, 9).

EXPERIMENTS

The experiments are divided into 6 groups. A total of 17 dogs and 29 rabbits was used.

Elimination of posterior pituitary deficiency as the cause of changes seen after hypophysectomy in the dog. In the first paper of these studies (8) it was shown that hypophysectomy in the dog resulted in a rise of serum amylase activity. It was suggested, but not proved, that this change in amylase activity was due to removal of the anterior lobe. Posterior pituitary secretion, however, is known to exert an effect on carbohydrate metabolism. Pituitrin raises the blood sugar and is antagonistic to insulin (10). It was, therefore, possible that some, if not all, of the rise following hypophysectomy was due to removal of the posterior lobe.

During an operation by the parapharyngeal approach (11) on the pituitary of a large female dog, a fortuitous opportunity to study this question presented itself (*dog 1*). While delivering the pituitary the anterior lobe pulled away from the posterior lobe and fell back into the cranial cavity. Only the posterior lobe and the infundibular stalk were removed. It was not accurately determined at operation what blood supply remained intact to the anterior lobe tissue. At autopsy 6 months later the anterior lobe, grossly normal, lay firmly rooted in the shallow sella turcica. Thin adhesions connected the anterior lobe to the infundibular region. From the behavior of the dog after operation it is probable that the lobe was left attached by a strip of pia mater furnishing some blood supply and that the gland did not survive merely as a graft.

After operation diabetes insipidus appeared immediately and was permanent. In the 6th postoperative month the diabetes was as severe as earlier; therefore presumably no regeneration of pituitrin secreting tissue occurred. The daily urinary volume varied usually in waves from 2000 to 4500 cc., with occasional swings as low as 500 and as high as 6800 cc. In the 3rd and again in the 5th postoperative months, 8-day periods of fast with water were instituted; although the polyuria fell off slowly, the blood sugar remained normal throughout. The absence of spontaneous hypoglycemia offered physiological proof of intact anterior lobe tissue. The insulin sensitivity was, however, increased above the average for the normal dog. (It was not observed preoperatively.) This suggested a possible partial anterior

lobe deficiency although it may have been due in part to the absent posterior lobe mechanism.

The serum amylase activity of this dog was closely followed before and for the first 7 weeks after operation. No change from the normal occurred.

Effect of anterior pituitary extract. Two normal male dogs (no. 2, 3) were each injected intraperitoneally with a large dose of anterior pituitary extract. The extract was made from fresh steer and cow anterior pituitary lobes placed immediately in carbon dioxide snow. The procedure of extraction as outlined by Young (12) was followed.

The data of *dog 2* are shown in figure 1. The blood sugar rose to a peak during the first 48 hours after injection; the serum amylase activity declined during the same period. By 72 hours the blood sugar had fallen nearly to normal; the amylase activity had risen during this period to a level above the pre-injection control. For the next 6 days the amylase activity showed unusual fluctuations.

In the second animal, *dog 3*, weight 13.3 kg., the extract from a total of 105 gm. of anterior pituitary tissue was injected in multiple doses. The effect upon the blood sugar and serum amylase activity was the same as in *dog 2*. The curves of both dogs ran closely parallel except that the lowest point on the amylase curve, 8.0 mg. (5% serum), was reached in 24 hours while the peak of the blood sugar, 133 mg., was not reached until 72 hours. By the 6th day after the start of the injection the amylase was 2.3 mg. above the pre-injection control of 12.0 mg. By the 8th day after injection both the amylase activity and blood sugar had returned to normal.

It is believed that the primary effect of the anterior pituitary extract on the serum amylase activity is to depress it. The secondary overshooting is probably the result of a compensatory mechanism and is comparable to that seen after insulin in the normal animal (see below).

Effect of hypophysectomy in the rabbit. Following bilateral adrenalectomy in the rabbit, the blood serum amylase activity either remained unchanged or decreased slightly. In view of this striking contrast to the change seen in the adrenally insufficient dog, it was decided to study the amylase activity in the hypophysectomized rabbit.

The data of 9 hypophysectomized rabbits are shown in figure 2. In 3 animals, no. *58*, *72*, *89*, there was a slight rise in activity after operation. The survival period of these 3 animals was less than 5 days. In all the remaining animals there was a moderate drop in activity.

The pancreatectomized-hypophysectomized dog. The finding of Markowitz and Hough (4) and Reid, Quigley and Myers (5) that the serum amylase activity of the depancreatized dog falls in diabetic acidosis and returns to normal with insulin administration has been confirmed with 5 dogs in this laboratory. In view of the rise in amylase activity after removal of the pituitary (8) it seemed desirable to weigh the result of forces existing after removal of both glands.

The data obtained from a depancreatized, and later hypophysectomized, animal, *dog 4*, are shown in figure 3. Following pancreatectomy the animal was maintained on insulin through 11 days. Insulin was then omitted and

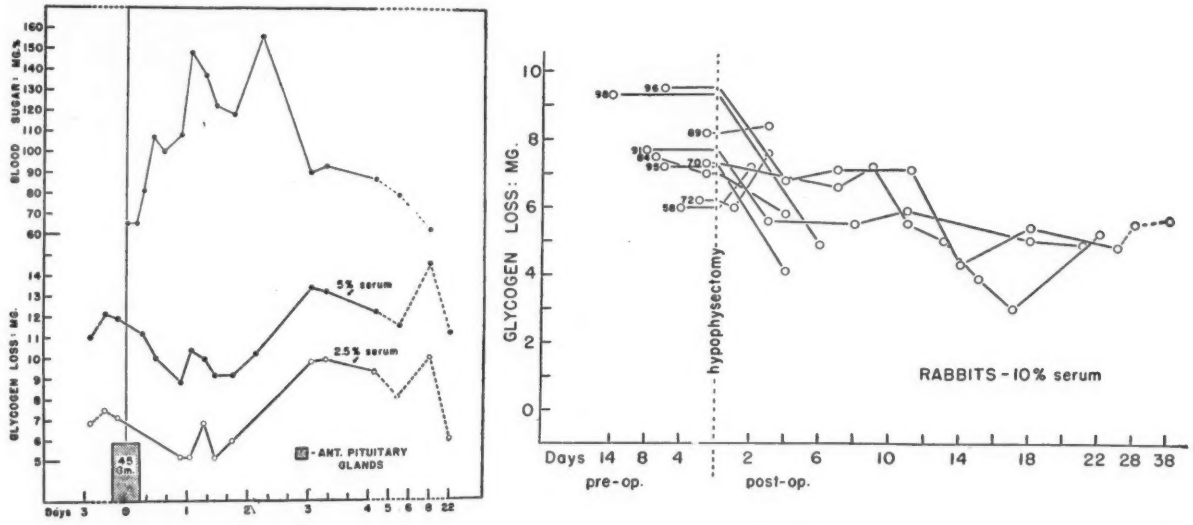


Fig. 1 (left). *Dog. 2*, male, weight 16.0 kg. ANTERIOR PITUITARY EXTRACT. Blood sugar; blood serum amylase activity, 5 and 2.5% serum concentrations. Extract made from 45.5 gm. of ox anterior lobe tissue. Urine showed neither ketone bodies nor sugar at the height of glycemia. Fig. 2 (right). HYPOPHYSECTOMIZED RABBITS. Blood serum amylase activity, 10% serum concentration.

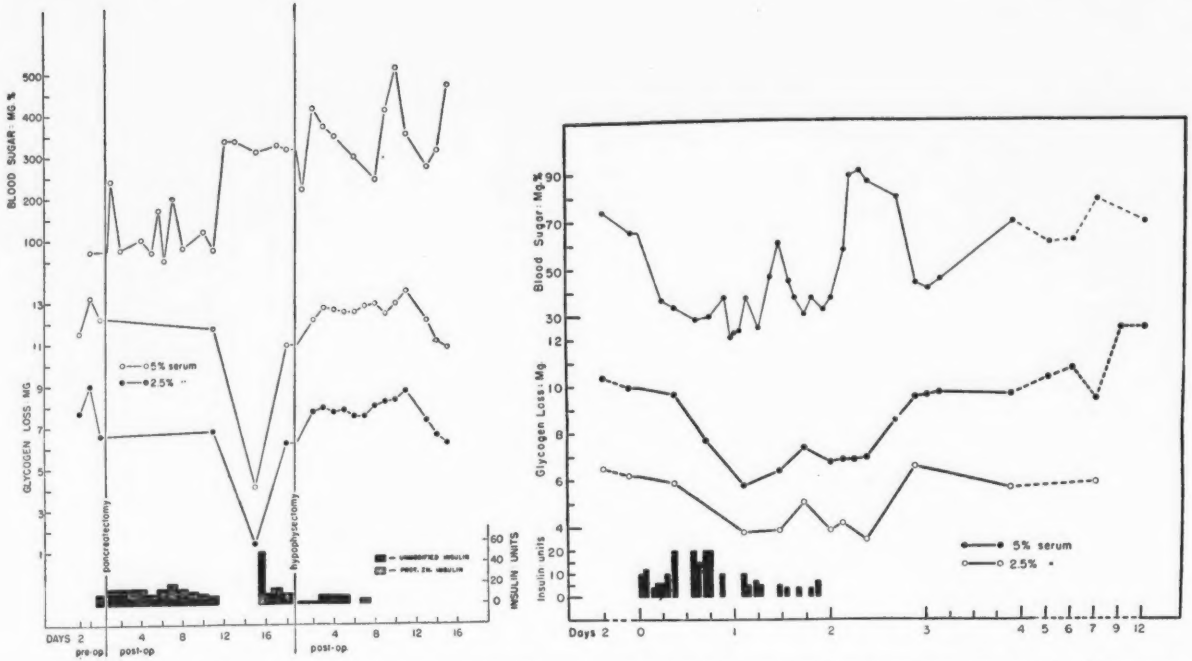


Fig. 3 (left). *Dog 4*, male, weight 10.8 kg. PANCREATECTOMIZED-HYPOPHYSECTOMIZED. Blood sugar; blood serum amylase activity, 5 and 2.5% serum concentrations; insulin dosage. Fig. 4 (right). *Dog 5*, male, weight 16.0 kg. INSULIN IN NORMAL DOG. Blood sugar; blood serum amylase activity, 5 and 2.5% serum concentrations; insulin dosage.

the animal allowed to slide into typical acidosis. The expected drop in serum amylase activity occurred. Following the correction of the diabetic acidosis with insulin the amylase activity returned to normal. The dog was then hypophysectomized (11). During the first 7 post-hypophysectomy days, small doses of insulin were added to the daily intravenous injections of saline and glucose. The animal was clinically doing well on the 15th day when the last blood determinations were made. Besides the blood sugar and amylase determinations, the N.P.N. was 32 mg., the serum chloride 110 m.eq., sodium 141.6 m.eq. and protein 5.2 gm.%. The animal died an undetermined number of hours later, exact cause unknown. Postmortem examination

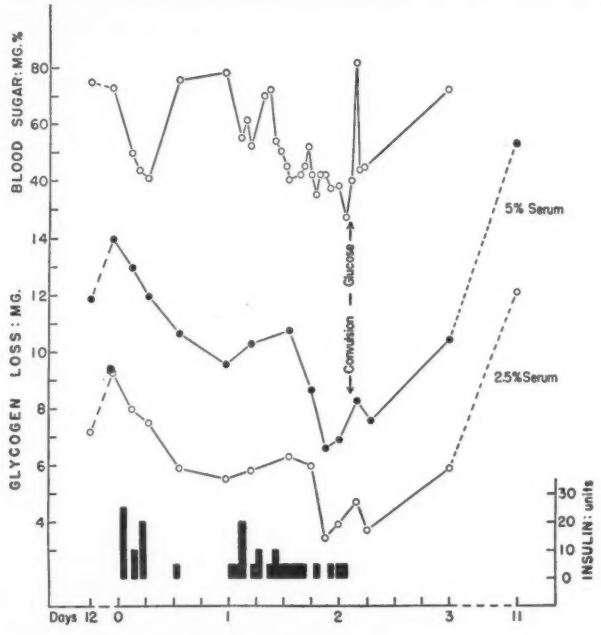


Fig. 5. *Dog.* 6, female, weight 29.0 kg. INSULIN IN NORMAL DOG. Blood sugar; blood serum amylase activity, 5 and 2.5% serum concentrations; insulin dosage.

showed grossly complete removal of both glands, and other findings were negative. During the period following the second operation the blood sugar had shown the tremendous fluctuations characteristic of these Houssay animals. Although the serum amylase activity varied, all readings were within the limit of the normal range for dogs; neither a fall characteristic of acidosis nor a rise typical of pituitary insufficiency occurred.

Effect of insulin in the normal dog and rabbit. In the first paper of this series (8), the finding previously reported by others that insulin injected into the normal animal had no effect on serum amylase activity was confirmed. Upon reconsideration of the changes following hypophysectomy this seemed to be an unreasonable finding. The idea occurred that the action of

insulin, or hypoglycemia, had not existed for a long enough period to create a significant change in serum amylase activity.

Accordingly, 8 norml dogs were injected with repeated doses of insulin sufficient to produce hypoglycemia for 48 hours or longer. In all animals there was a fall in serum amylase activity. A significant fall, however, did not occur until hypoglycemia had existed for 6 hours. If the hypoglycemia was maintained, the slow fall in amylase activity continued until a level nearly as low as that seen in diabetic acidosis was reached. Insulin withdrawal was followed by a slow rise, again following a latent period of 6 or more hours. In 4 animals insulin was withdrawn after 48 to 72 hours. In 3 of these a transient rise to above the pre-injection level occurred in from one to 11 days.

In figure 4 the data of *dog 5* are shown. The latency of the fall and rise of the serum amylase activity behind the blood sugar curve is clearly seen.

In figure 5 the data of *dog 6* are shown. It is seen that the lowest amylase activity was not reached until the end of the second day and until a blood sugar level around 40 mg. had been maintained for several hours. This animal was subjected to a second more prolonged experiment with insulin 6 weeks later. A similar fall in amylase activity took place which was maintained until death in hypoglycemia occurred on the seventh day.

Four more of the dogs were continued in hypoglycemia until death occurred between 8 and 14 days. In 3 of these a diminished amylase activity was maintained until death. In the fourth there was an abrupt rise just before death to a high level of activity comparable to that seen in terminal adrenal insufficiency. The origin of this terminal rise is not clear. It may have been due to convulsions, prolonged cerebral anoxemia, or glandular failure elsewhere. In one of the 2 dogs surviving until the 14th day, grossly enlarged adrenal cortices were found at autopsy.

Hypoglycemia was similarly produced in 4 rabbits for 24 to 48 hours. In 2 there was a decrease of 2 mg. loss in serum amylase activity; in the other 2 no change occurred.

Effect of thyroidectomy and the thyroid hormone in the dog and rabbit. A large male dog was thyroidectomized, carefully preserving the outer parathyroids with intact blood supply. In the next 3 months the serum amylase activity varied in two waves to points below and above the usual normal limits. The signs of myxedema appeared with an increase in weight from 29 to 35 kg.; the blood cholesterol was 298 mg.%. Desiccated thyroid was then given by mouth in doses up to 12 grains a day. The only change was that the serum amylase activity was more stable within the usual normal values; the animal's weight returned to 29 kg., and the cholesterol fell to 182 mg.%.

Eleven rabbits were thyroidectomized. Completeness of thyroidectomy was proven in all animals by a 40% drop in metabolic rate.[3] In 2 animals there was a slight rise in serum amylase activity; in one, consistently low values were obtained; in 8, no change occurred.

Four rabbits were given subcutaneous injections of thyroxin, averaging 1 mg. per day, for 11 to 13 days. The animals were observed for 12 to 17 days. The metabolic rates rose and the animals lost weight. Although there

[3] We are indebted to Dr. J. Lerman for supervising the metabolic rate determinations.

was a significant slight rise in serum amylase activity in one animal, no change occurred in the others.

DISCUSSION

It is possible that alterations in function of the acinar or externally secreting cells of the pancreas may result in changes of serum amylase activity. There can no longer, however, be any doubt that in the dog, at least, the internal secretion of the pancreas is concerned with this amylase system. It is also clear that those endocrine glands, which in other respects are known to be intimately related to carbohydrate metabolism, do influence serum amylase activity.

The experiments reported here do not throw any light on the chemical mechanism of activation and inhibition of this enzyme system. There are curious anomalies in the observations which remain to be explained. It is remarkable that insulin injected into the normal dog should decrease rather than increase the amylase activity. The decrease in activity following pancreatectomy and the rise after hypophysectomy fit in well with the known antagonism between the two glands. That anterior pituitary extract should cause a decrease in activity, the reverse of that seen after removal of the pituitary, is also to be expected.

The explanation of the behavior of insulin is probably as follows: when injected into the acidotic (insulin-insufficient) animal, a rise to normal occurs; when given in physiological excess to the normal animal, it, or the hypoglycemia produced, calls into action a compensatory mechanism, the net result being a decrease in serum amylase activity. When the insulin is withdrawn, over-compensation follows for a brief period. Overactivity of the compensatory mechanism also occurs following injection of anterior pituitary extract.

No light is thrown by these experiments upon the reason for the relative inactivity of serum amylase in the rabbit as compared with that in the dog. It might have been supposed that the rabbit, whose diet normally contains more carbohydrate than the dog's, would have had the more active amylase system. The observation that the changes in the rabbit following removal of the pituitary and adrenal are less striking than those in the dog, fits with the finding that the rabbit's amylase system is less active. There is no clue, however, as to why the rabbit's amylase system responds usually in the opposite direction. Both the normal rabbit and dog respond to insulin with a drop in amylase activity, and in both animals there is no consistent change with alterations in thyroid function; but here agreement ceases. The finding that the amylase activity is inclined to fall following both hypophysectomy and adrenalectomy in the rabbit, in contrast to the sharp rise seen in the dog, suggests that the serum amylase system is not directly influenced by any one of the hormones under consideration.

The experiments with insulin are a further demonstration of the greater ease of producing an effect by a hormone in an animal in which the gland responsible for the hormone has been ablated. A deficiency is readily corrected, while the equilibrium of the normal animal is difficult to disturb.

Alterations in serum amylase activity take place slowly in the body. This enzyme system is not a labile mechanism with rapid fluctuations from hour to hour. It is worthy of note that the long latent period between the onset of hypoglycemia and the appearance of change in amylase activity suggests that the enzyme system must be acted upon for several hours before a measurable change takes place.

SUMMARY

In a dog with intact anterior pituitary tissue and permanent diabetes insipidus from ablation of the posterior pituitary lobe and stalk, there was no change in blood serum amylase activity. Loss of the posterior lobe was, therefore, excluded as the source of the rise in amylase activity previously described following total hypophysectomy.

Injection of anterior pituitary extract in the normal dog causes a fall in serum amylase activity.

The fall in amylase activity in acidosis following pancreatectomy in the dog and the rise to normal with insulin administration is confirmed. In the pancreatectomized, hypophysectomized dog, the amylase activity remains within normal limits.

The absence of effect of insulin on the serum amylase of the normal animal is not confirmed. Insulin hypoglycemia, maintained for more than 6 hours, results in a decrease in activity in dogs and rabbits. If the hypoglycemia is maintained in the dog, the level reached in 36 hours is comparable to that seen in diabetic acidosis. The probable reason for this unexpected result is discussed.

Thyroidectomy and the thyroid hormone in the dog and rabbit produce as their only effect a greater variation of activity of the serum amylase system. No consistent variation or tendency was encountered.

Removal of the pituitary in the rabbit results in a slight fall in serum amylase activity in contrast to the rise seen in the hypophysectomized dog. The significance of the difference in behavior of the serum amylase systems of the dog and rabbit to loss of anterior pituitary and adrenal cortical hormones is discussed. It is suggested that the various hormones influencing the serum amylase system do so indirectly.

It is concluded that those endocrine glands which are intimately related to carbohydrate metabolism affect the serum amylase system.

REFERENCES

1. Elman, R.: *Ann. Surg.* 105: 379. 1937.
2. Friedman, I., and W. R. Thompson: *Ann. Surg.* 104: 388. 1936.
3. Karsner, H. T., H. L. Koeckert and S. A. Wahl: *J. Exper. Med.* 34: 349. 1921.
4. Markowitz, J., and H. B. Hough: *Am. J. Physiol.* 75: 571. 1926.
5. Reid, E., J. P. Quigley and V. C. Myers: *J. Biol. Chem.* 99: 615. 1933.
6. Scharles, F. H., P. D. Robb and W. T. Salter: *Am. J. Physiol.* 111: 130. 1935.
7. Hayes, D. R., and W. T. Slater: Medical Papers Dedicated to Dr. Henry A. Christian, February, 1936.
8. Cope, O., A. Hagström and H. Blatt: *Am. J. Physiol.* 122: 428. 1938.
9. Cope, O., I. Kapnick, A. Lambert, T. D. Pratt and M. G. Verlot: Endocrinology 25: 236. 1939.
10. Burn, J. H.: *J. Physiol.* 57: 318. 1923.
11. Cope, O. and G. A. Donaldson: *Endocrinology* 24: 475. 1939.
12. Young, F. G.: *Biochem. J.* 32: 513. 1938.

THE RECIPROCAL NATURE OF THE TESTIS-COMB RELATIONSHIP[1,2]

W. H. HOSKINS AND F. C. KOCH
From the Department of Biochemistry,
The University of Chicago
CHICAGO, ILLINOIS

ZAWADOWSKY (1) has reported that ground cocks' combs fed to young male rats produce smaller testes, seminal vesicles, and prostate glands than normal. He obtained the same result by the injection of alcohol extracts of comb tissue. Buckner, Insko, and Martin (2) have shown that comb removal in cockerels leads to larger testes and increased fertility. Zawadowsky confirmed the result of comb removal on testis size. From these and similar experiments Zawadowsky postulates a reciprocal relationship between secondary sex characters such as the comb and the gonads, in this case, the testis. According to this suggestion, not only does the testis stimulate the comb, the accepted relationship, but the comb in turn inhibits the growth of the testis and in this way controls its output of male hormone. Such a regulatory mechanism would have many challenging aspects and would involve a reconsideration of accepted mechanisms of sexual physiology. We have repeated Zawadowsky's experiments on comb feeding, comb-extract administration, and comb removal and extended them in some aspects in an effort to determine the existence of such a relationship.

The Effect of Comb Tissue Fed to Male Rats

We were unable to confirm Zawadowsky's findings as to the effects of feeding comb tissue, fresh or dried, to young male rats. Groups of rats ranging from 3 to 14 weeks of age were fed 6.0 to 12.0 gm. of fresh comb tissue, or its equivalent of dried tissue, per day for periods of 4 to 13 weeks (table 1). In every experiment we found slightly larger testes, seminal vesicles, and prostates in the comb-fed animals than in the litter-mate controls, in contrast to the effect reported by Zawadowsky. The average testes-weight/body-weight ratio for our 5 experiments, using 49 experimental and 58 control rats, was 1.20% for the controls and 1.29% for the comb-fed rats, a difference of 0.09% in favor of the comb-fed rats. Zawadowsky's experiments, using 21 experimental and 16 control rats, when calculated on this basis, give testis/body-weight ratios of 1.22% for the controls and 1.14% for the comb-fed rats, a difference of 0.08% in favor of the controls. These differences, equal in magnitude but opposite in direction, may be due to dietary factors. Zawadowsky fed part of his control animals chicken meat plus the stock diet, another group of controls were fed chicken feet plus the stock diet, and a third group received only the stock diet. The experimental animals also received the stock diet plus comb tissue, but the controls were given only the stock diet (Purina Dog Chow, milk, bread, carrots). In our experi-

[1] These investigations were supported in part by a grant from the Rockefeller Foundation.
[2] We are indebted to Mr. Paul Mandeville of the Associated Poultry Industries for the comb tissue.

ments, the comb-fed animals probably received a slightly better diet than the controls while in Zawadowsky's experiments the control animals may have had the more adequate diet. The gonads are known to be sensitive to slight changes in body

TABLE 1. COMB TISSUE FED TO YOUNG MALE RATS (EXPERIMENTS 1 TO 5)

Exp.	Age	Length of exp.	Fed daily	Number of rats		Testis weight		Test wt. / Body wt.		Sem. ves. weight		S. V. wt. / Body wt.		Prostate weight		Pros. wt. / Body wt.	
				Con.	Exp.	Con.	Exp.	Con.	Exp.	Con.	Exp.	Con.	Exp.	Con.	Exp.	Con.	Exp.
	days					gm.	gm.	%	%	mg.	mg.	%	%	mg.	mg.	%	%
1	108	30 da.	1.0 gm. dried (=6.0 gm. fresh)	10	10	2.49 (1.88 to 2.96)	2.60 (2.04 to 2.82)	1.07	1.15	323 (60 to 570)	420 (210 to 753)	0.139	0.186	176 (36 to 388)	179 (76 to 253)	0.076	0.079
2	21–28	4 wk.	6.0 gm. fresh	10	8	1.36 (0.28 to 2.01)	1.67 (0.58 to 2.31)	1.21	1.26	26 (5 to 51)	40 (9 to 112)	0.023	0.030	46 (4 to 86)	65 (23 to 139)	0.041	0.049
3	21	3 mo.	6.0 gm. fresh	19	13	2.65 (2.46 to 2.93)	2.93 (2.44 to 3.39)	1.24	1.31	560 (270 to 810)	630 (460 to 790)	0.262	0.283	160 (100 to 220)	210 (150 to 260)	0.075	0.094
4	28	4 wk.	6.0 gm. fresh	9	8	1.49 (0.30 to 2.38)	1.62 (0.58 to 2.14)	1.16	1.38	36 (4 to 89)	30 (5 to 55)	0.028	0.026	53 (8 to 108)	50 (81 to 86)	0.041	0.043
5	26	25 da.	2.0 gm. dried (=12.0 gm. fresh)	10	10	1.41 (1.02 to 2.10)	1.53 (1.12 to 2.05)	1.33	1.35	29 (12 to 65)	31 (10 to 80)	0.027	0.027	45 (26 to 92)	49 (23 to 73)	0.042	0.043
Total				58	49												
Average								1.20	1.29			0.096	0.110			0.055	0.061

condition and the differences in diet in these experiments may have been enough to cause the slight testis effect observed.

The Effect of Comb Tissue Fed to Cockerels

If comb tissue contains a substance which affects the testis it might be expected to act more readily on the testis of the cockerel than of the rat. Dried comb tissue was fed to normal cockerels, administering the equivalent of 3.0 to 12.0 gm. of fresh comb-tissue per day in gelatine capsules for periods of 4 to 13 weeks. Six experiments, using a total of 57 comb-fed and 56 control cockerels showed no consistent effects of the comb feeding (table 2). There were some indications of a slight increase in body weight, testis-weight/body-weight ratio, and comb growth, but the differences were small and inconsistent. The absence of an inhibitory effect is in agreement with the experiments on rats.

The Effect of Comb Tissue Fed to Capons

The presence of an active substance in comb tissue was tested for in another way by feeding dried comb tissue—the equivalent of 3.0 gm. of fresh comb per day—to capons for 4 weeks. Any effect of the feeding of comb tissue on the capon comb itself could be detected by its effect on the response to male hormone. The comb-growth response of these capons to injections of androsterone was tested before and after the 4 weeks of comb feeding, but no significant difference was found (table 3).

These 3 types of experiments, feeding comb tissue to rats, cockerels, and capons, give no evidence to support the theory of an action of comb tissue on the testis or secondary sex organs.

TABLE 2. COMB TISSUE FED TO COCKERELS (Experiments 6 to 10)

Exp.	Age	Length of exp.	Fed daily	Number of cockerels		Comb weight		Comb wt. Body wt. ×100		L+H of comb before		L+H of comb after		Comb change		Testes weight		Testes wt. Body wt. ×100	
				Con.	Exp.	Con.	Exp.	Con	Exp.	Con.	Exp.	Con.	Exp.	Con.	Exp.	Con.	Exp.	Con.	Exp.
6	2 mo.	44 da.	0.5 gm. dried (⇔3.0 gm. fresh)	10	7	gm. 6.5 (2.1–11.9)	gm. 15.3 (7.9–27.2)	% 0.785	% 1.66	mm.	mm.	mm.	mm.	mm.	mm.	gm. 0.35 (0.25–0.50)	gm. 0.91 (0.42–2.18)	% 0.0423	% 0.0921
7A	1 mo.	30 da.	0.5 gm. dried	13	12					13.2 (10.2–15.6)	13.2 (11.0–15.9)	13.7 (10.7–16.9)	14.0 (9.8–17.2)	0.5	0.8	0.65 (0.28–2.93)	0.55 (0.35–0.97)	0.0556	0.0478
7B			1.0 gm. dried (⇔6.0 gm. fresh)		10						13.0 (10.5–15.7)		14.7 (11.9–17.0)		1.7		0.76 (0.45–2.81)		0.0650
8	1 mo.	7 wk.	1.0 gm. dried	9	7					5.7 (4.5–7.3)	5.6 (4.2–6.3)	17.2 (14.7–21.2)	16.4 (14.7–17.9)	11.5	10.8	6.56 (1.05–11.31)	6.59 (3.30–(8.90)	0.547	0.579
9	1 mo.	13 wk.	1.0 gm. dried	6	8					5.6 (3.9–7.5)	5.5 (4.4–6.7)	17.7 (15.5–21.8)	20.2 (16.7–26.9)	12.1	14.7	6.54 (1.83–15.54)	14.82 11.05–22.78)	0.60 (±0.46)	0.98 (±0.20)
10	2 wk.	30 da.	2.0 gm. dried (⇔120 gm. fresh)	18	13											0.117 (0.031–0.360)	0.096 (0.050–0.153)	0.039	0.035
Totals				56	57														

The Injection of Comb Extracts into Rats

The injections of alcohol extracts of comb tissue into 3 or 4-week-old rats produced, however, a decreased body weight, testis, seminal vesicle, and prostate weight (table 4). These results are in agreement with those of Zawadowsky. The quantity of extract injected was equivalent to 6.0 to 23.0 gm. of fresh comb tissue per day for 3 to 4 weeks. Five experiments, using a total of 40 experimental and 38 control rats showed an average difference in final body weight of 23.0 gm. between the injected and the control rats. The testis-weight/body-weight ratio was 1.30% for the controls and 0.88% for the injected animals while the seminal vesicle/body-

TABLE 3. EFFECT OF COMB-TISSUE FEEDING ON THE COMB-GROWTH RESPONSE OF CAPONS

	Number	Comb fed	Comb growth before experiment[1]	Comb growth after experiment[1]	Difference
			mm.	*mm.*	*mm.*
Controls	10	—	6.7	6.6	−0.1
Comb-fed	10	≑ of 3.0 gm./day for 4 wk.	5.5	5.9	0.4

[1] Comb-growth response to 0.1 mg. androsterone for 5 days determined by method of Gallagher and Koch (3).

weight ratios were 0.025% and 0.014% respectively, and the same ratio for the prostate, 0.045% and 0.028% in the controls and experimentals. Zawadowsky does not state the amount of tissue represented by the extracts used in his experiments but since the effects were less severe than in ours it is reasonable to assume that smaller concentrations were used. We also observed a very slight inhibition of testis growth when alcohol extracts of comb tissue were fed to young male rats by stomach syringe (table 5). These rats were given the same dose of comb extract for the same length of time as in the injection experiment where the most striking results were obtained but the inhibiting effect on the testis was not so marked.

It is clear that alcohol extracts of comb tissue produce a definite slowing of the rate of body growth as well as decreased testis, seminal vesicle, and prostate size in young male rats. It must be pointed out, however, that these experiments give no indication as to whether or not this inhibition operates in the normal cockerel, as postulated by Zawadowsky, or whether it is merely a type of injury due to the administration of foreign substances, in this case a mixture of phospholipins. The signs of toxicity which appeared immediately after each injection as convulsions and general pain reactions and later by the marked loss in body weight, the presence of nose-rot and weakness, are similar to the observed effects of the injections of phospholipin mixtures in rats (4). The testis injury may be similar to that produced by a variety of substances: fuel oil, carbon monoxide, testis tissue, and others (5, 6, 7, 8). The loss of body weight accompanying the small testes in the injected animals prevents us from assuming that a specific inhibiting action on the testis is present here.

The Effect of Alcohol-Extracted Comb-Tissue Fed to Rats

In order to check the possibility that alcohol extraction had removed toxic materials from the comb tissue and left the hypothetical active substance behind, we

TABLE 4. ALCOHOL EXTRACTS OF COMB TISSUE INJECTED INTO MALE RATS (Experiments 12 to 15)

Exp.	Age of rats	Length of exp.	Quantity injected daily	Number of rats		Body wt. gain		Testes wt.		Testis wt. / Body wt.		Seminal vesicle wt.		Sem. ves. wt. / Body wt.		Prostate wt.		Prostate wt. / Body wt.	
				Con.	Exp.	Con.	Exp.	Con.	Exp.	Con.	Exp.	Con.	Exp.	Con.	Exp.	Con.	Exp.	Con.	Exp.
12A	25 da.	25 da.	≏ of 6.0 gm.	9	9	gm. 60	gm. 65	gm. 1.63 (1.06–2.08)	gm. 1.59 (0.67–2.20)	% 1.54	% 1.36	mg. 27 (13–38)	mg. 23 (7–35)	% 0.026	% 0.019	mg. 56 (26–82)	mg. 54 (21–(87)	% 0.053	% 0.045
12B			≏ of 12.0 gm.		6		30		0.55 (0.14–0.98)		0.66		9 (4–14)		0.010		21 (8–42)		0.026
13	28 da.	4 wk.	≏ of 6.0 gm.	9	8	78	45	1.49 (0.30–2.38)	0.68 (0.14–1.59)	1.16	0.72	36 (4–89)	10 (4–31)	0.028	0.011	53 (8–108)	15 (5–36)	0.014	0.016
14	26 da	25 da	≏ of 12.0 gm.	10	10	64	50	1.41 (1.02–2.10)	1.17 (0.40–1.71)	1.33	1.17	29 (12–65)	20 (8–39)	0.027	0.020	45 (26–92)	36 (20–60)	0.042	0.036
15	21 da.	21 da.	≏ of 23.0 gm.	10	7	60	21	1.22 (0.84–1.64)	0.30 (0.14–0.58)	1.18	0.51	19 (9–45)	5 (3–7)	0.018	0.009	46 (36–66)	9 (4–15)	0.045	0.015
Total				38	40					1.30	0.88	28	13	0.025	0.014	50	27	0.045	0.028
Average						65	42	1.44	0.86										

fed the extracted tissue to rats under conditions comparable to those used in the feeding of whole comb tissue, as far as age of rats, dose of tissue, and length of feeding was concerned (table 6). No effect on body weight, testis, seminal vesicle, or prostate size was found.

TABLE 5. ALCOHOL EXTRACT OF COMB TISSUE FED TO MALE RATS
(equivalent of 23.0 gm. comb tissue per day fed for 21 days to 21-day-old rats)

	Number of rats	Weight gain	Testes wt. / Body wt.	Sem. ves. wt. / Body wt.	Prostate wt. / Body wt.
		gm.	%	%	%
Controls	10	60	1.18	0.0185	0.045
Extract-fed	9	58	1.04	0.016	0.039

The Effect of Comb Removal in Cockerels

The failure of feeding experiments with comb tissue to produce evidence of testis-inhibition and the doubtful significance of the inhibition obtained with extracts of comb tissue does not rule out the possibility that a reciprocal testis-comb relationship may exist, although these experiments seem to invalidate a part of the supporting evidence cited by Zawadowsky. Inhibiting substances might be secreted by the comb in such low concentrations that the relatively crude tests employed here would fail to detect them. A more physiological approach to the problem is the removal of the comb from cockerels and the determination of any effects on the organism. The experiments of Buckner, Insko, and Martin (2) and of Zawadowsky (1) already referred to, indicate that comb removal leads to the production of larger testes than normal within 3 months (1) or 6 months (2) after removal. This effect, if significant, is indicative of an actual testis-inhibiting or regulating mechanism controlled by the comb.

We performed 4 experiments on the effect of comb removal in cockerels using 43 normal controls and 42 combless cockerels (table 7—experiments 21 to 24). The combs and wattles were removed by an electric cautery at 2 to 8 weeks of age and the birds were killed after 1 to 3 months. No consistent effects on body weight or testis size were found after this length of time. After their completion we were informed of similar experiments carried out by Marlow and co-workers and these results were made available to us through the kindness of Dr. Marlow (9). The findings of these workers are in agreement with ours in showing no effect of comb removal on testis size after 3 months. When, however, Marlow extended the time after comb removal to 7 months or more, a marked effect on testis size was apparent. We repeated our previous experiments, removing the combs and wattles at 3 weeks of age and killing the animals 7 months later and confirmed Marlow's findings (table 7—experiment 25). The body weights of 13 normal controls increased 1473 gm. during the 7-month period while the 12 combless animals gained an average of 1553 gm. The average testis weights were 3.14 gm. for the controls and 11.04 gm. for the combless birds, expressed as per cent of body weight the testis values are 0.19% and 0.65% for the controls and combless birds respectively.

The significant effect of comb removal on testis size apparent 7 months after comb removal seems to indicate a true action of the comb on the testis.

TABLE 6. ALCOHOL-EXTRACTED COMB TISSUE FED TO MALE RATS (Experiments 17 to 19)

Exp.	Age of rats	Length of exp.	Quantity fed daily	Number of rats		Body wt. gain		Testes wt.		Testes wt. / Body wt.		Seminal vesicle wt.		Sem. ves. wt. / Body wt.		Prostate wt.		Prostate wt. / Body wt.	
				Con.	Exp.	Con.	Exp.	Con.	Exp.	Con.	Exp.	Con.	Exp.	Con.	Exp.	Con.	Exp.	Con.	Exp.
17	25 da	25 da	⇦ to 6.0 gm. fresh comb	6	9	gm. 60	gm. 45	gm. 1.63 (1.06–1.08)	gm. 1.27 (0.64–2.32)	% 1.54	% 1.29	mg. 27 (13–38)	mg. 71 (6–44)	% 0.026	% 0.016	mg. 56 (26–82)	mg. 38 (17–77)	% 0.053	% 0.035
18	28 da	4 wk.	⇦ to 6.0 gm. fresh comb	9	9	78	77	1.49 (0.30–2.38)	1.61 (0.41–2.42)	1.16	1.29	36 (4–89)	38 (5–113)	0.028	0.030	53 (8–108)	51 (9–89)	0.041	0.041
19	26 da.	25 da.	⇦ to 12.0 gm. fresh comb	9	10	64	69	1.41 (1.02–2.10)	1.69 (1.24–2.01)	1.33	1.42	29 (12–65)	36 (18–94)	0.027	0.030	45 (26–92)	58 (31–110)	0.042	0.049
Average Totals				24	28	67	64	1.51	1.52	1.34	1.33	31	30	0.027	0.025	51	49	0.045	0.042

An attempt was made to check this in one of the early experiments. Comb tissue was fed to one group of combless cockerels, in parallel with the 3 month comb-removal experiments. Since, however, no effect of comb removal could

TABLE 7. EFFECT OF COMB REMOVAL IN COCKERELS (Experiments 21 to 25)

Exp.	Age at start	Time after removal	Number used		Weight change		Testes weight		Testes weight / Body weight	
			Con.	Exp.	Con.	Exp.	Con.	Exp.	Con.	Exp.
21	2 mo.	44 da.	10	8	gm. 272	gm. 225	gm. 0.35 (0.24–0.50)	gm. 0.56 (0.39–1.12)	% 0.0423	% 0.0732
22	1 mo.	7 wk.	9	9	922	820	6.56 (1.05–11.31)	5.11 (0.25–10.37)	0.547	0.436
23	1 mo.	13 wk.	5	5	898	748	6.54 (1.83–15.54)	2.97 (0.55–9.54)	0.60	0.23
24	2 wk.	30 da.	18	20	300[1]	310[1]	0.117 (0.031–0.360)	0.116 (0.054–0.297)	0.039	0.037
25	1 mo.	7 mo.	13	12	1473	1553	3.14 (0.9–8.6)	11.04 (1.0–21.8)	0.194	0.64

[1] Final weight.

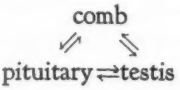
be detected after this interval the lack of testis effect in the comb-fed, combless cockerels has no significance. Comb feeding to combless cockerels for seven months or longer is necessary to establish this point.

Effect of Light on Testis Size in Normal and Combless Animals

Testis and comb size are known to depend upon the extent of exposure to light (2). We attempted to compare this effect in normal and in combless cockerels under normal light conditions and in almost complete darkness over a period of 7 months. The data from the birds in normal light has been discussed (experiment 25). The birds kept in the dark, however, were weak and almost all died before the end of the experiment. This experiment needs to be repeated under less drastic conditions in order to determine the place of the comb in the relationship between light intensity and testis size.

Although the length of time necessary to produce an effect on testis size after comb removal indicates a very slight degree of activity on the part of the comb, its significance in an understanding of the sexual physiology of the cockerel is nevertheless important. The accepted rôle of the comb as a passive receptor in the relationship between the pituitary, testis, and comb must apparently be revised. Using Zawadowsky's scheme of expressing this reciprocal relationship, it becomes: pituitary⇌testis⇌comb, instead of pituitary⇌testis→comb. The part played by the pituitary and the possible

influence of light on this action of the comb require investigation before the mechanism can be interpreted. Such investigation may reveal that a triangular, reciprocal relationship exists, rather than the type of relationship suggested above. This might be expressed:



The recent finding that estrogens act directly on the comb, inhibiting its growth (10, 11, 12) may have to be considered in explaining the function of the comb in sex physiology.

SUMMARY

The experiments of Zawadowsky on feeding cockerels' combs to rats, injecting alcohol extracts of comb tissue into rats, and removing the combs and wattles of cockerels were repeated and extended. We could not confirm Zawadowsky's report that feeding comb tissue to rats damages the testes and accessory sex organs nor were we able to find any effects of feeding comb tissue to cockerels. Injections of crude alcoholic extracts of comb tissue produced testis and accessory sex-organ damage in rats, as claimed by Zawadowsky, but also produced a marked slowing of body growth and other evidences of toxicity, indicating a general toxic effect of the comb extract and not a specific inhibiting action on the testis. We found no effect on the testis weights of combless cockerels, 3 months after comb removal, in contrast to Zawadowsky. Seven months after comb removal, however, the testes of combless cockerels were significantly larger than those of normal controls. Part of the evidence cited by Zawadowsky in postulating a reciprocal testis-comb relationship is apparently faulty, but the existence of the relationship is indicated by the comb-removal experiments. The ability of the comb, a secondary sex organ, to influence the testis indicates the necessity for a reexamination of the accepted mechanisms operating in the sexual physiology of the fowl.

REFERENCES

1. Zawadowsky, M. M.: *Bull. de Biol. et Med. exper.* 1: 196. 1936; Personal communication.
2. Buckner, G. D., W. M. Insko and J. H. Martin: *Poultry Sci.* 71: 365. 1934.
3. Gallagher, T. F., and F. C. Koch: *J. Pharm. & Exper. Therap.* 55: 97. 1935.
4. Moore, C. R.: *Proc. Second Internat. Congr. for Sex Res.* 293. 1930.
5. Buchheim, V.: *Comp. rend. Soc. de Biol.* 108: 1210. 1931.
6. Williams, I. R., and E. Smith: *Am. J. Physiol.* 110: 611. 1935.
7. Myers, H. B., B. Vidgoff and W. C. Hunter: *Proc. Soc. Exper. Biol. & Med.* 30: 1099. 1933.
8. Koch, F. C.: *Physiol. Rev.* 17: 153. 1937.
9. Marlow, H. W.: Personal communication.
10. Regnier, V.: *Compt. rend. Soc. de Biol.* 127: 519. 1938.
11. Mühlbock, O.: *Acta brev. Neerl.* 8: 2/3, 50. 1938.
12. Hoskins, W. H., and F. C. Koch: Unpublished.

THE INHIBITION OF COMB GROWTH IN COCKERELS AND CAPONS BY ESTRONE[1,2,3]

W. H. HOSKINS AND F. C. KOCH

From the Department of Biochemistry, The University of Chicago

CHICAGO, ILLINOIS

ANTAGONISTIC as well as augmentative effects of the simultaneous administration of male and female sex hormones in mammals have been the subjects of numerous reports in the literature. The antagonistic effect is usually interpreted as an inhibition of the gonadotropic activity of the pituitary as suggested by Moore and Price (1), while the synergistic effects have not as yet been explained.

In Brown Leghorn capons, Juhn, D'Amour and Womack (2) found neither an antagonism nor an intensification of effect when purified testis-tissue concentrates (3 capon units per day) and a urinary estrogen preparation (150 R.U. per day) were injected simultaneously for 34 days. Juhn, Gustavson and Gallagher (3) administered urinary estrogen concentrates (10 R.U. per 25 gm. body weight) to young cockerels of various ages and found no evidence of antagonism except a shrinkage of the comb in a few birds in the group 12 weeks of age. Since the testes of these birds were normal histologically, the latter authors say: "the condition of the comb is then interpreted as due to some as yet unexplained physiological factor, and not as resulting from a suppression of the endocrine function of the male gonad." As these cases were exceptions, however, and the majority showed no such shrinkage, they conclude: "the female hormone in the dosages used was negative in . . . head furnishings of either sex."

The failure of hormone treatment in the fowl to afford any evidence of antagonistic or synergistic effects is difficult to reconcile with the striking effects obtained in mammals. This failure may be ascribed either to a fundamental species difference or to the presence of higher threshold values for sex-character reactivity in the fowl. This latter possibility we investigated by using large amounts of the pure hormones which were not available for the earlier work. Since the growth of the comb is one of the most sensitive and reliable indicators of male-hormone action, the effect of female hormone upon this response furnishes a good approach for a reexamination of one phase of sex-hormone antagonism in the fowl.

Our experimental approach was to administer intramuscularly crystal-

[1] These investigations were supported in part by a grant from the Rockefeller Foundation.

[2] The estrone used was supplied through the kindness of Dr. Cartland of the Upjohn Company, Kalamazoo, Michigan.

[3] The androsterone was supplied through the kindness of Dr. Schwenk of the Schering Corporation, Bloomfield, New Jersey.

line estrogenic and androgenic compounds in oil solution and to observe the reaction of the head furnishings. We used normal cockerels, capons, and incompletely castrated cockerels of the single comb White Leghorn strain.

The Effect of Estrone Administration on the Comb Growth of Incompletely Castrated Cockerels

Six incompletely castrated cockerels were used in the first experiment (4/27/37 to 8/9/37). Both testes had been removed at 4 to 6 weeks of age while preparing a large number of capons for male-hormone assays. The large combs and wattles present 2 months later indicated that residual or accessory testis tissue was present and secreting male hormone. The rate of comb growth was determined during a period of 7 weeks by measuring the increases in length and height. The birds were then divided into 2 groups comparable in initial comb size and rate of comb growth. Group 1 was injected with 0.5 mg. of estrone in 1 cc. of sesame oil daily for 3 weeks. Group 2 was not injected. The combs were measured each week. The combs of the uninjected birds continued to grow, the 3 incomplete castrates in this group showing increases of 27, 20, and 12 mm., with an average growth of 20 mm. In striking contrast the combs of the group injected with estrone not only failed to grow but in 2 of the 3 cases actually regressed significantly. The changes in comb size were -7, -8, and $+4$ mm., with an average decrease of 3.7 mm.

On ceasing injections, the estrone-treated group immediately resumed comb growth and in the course of the following weeks showed increases of 8, 7, and 4 mm., averaging 6.3 mm. During the same interval the uninjected control group continued the previous rate of comb growth with increases of 10, 6, and 4 mm., with an average increase of 6.7 mm.

As a confirmatory experiment, both to control the variability inherent in the small number of animals used and to obtain additional information on the resumption of comb growth in the group injected with estrone, we repeated our observations with the groups reversed—that is, Group 1 previously injected with estrone was now used for controls and the previous controls were injected with 0.5 mg. estrone per day for 3 weeks. The 3 incomplete castrates receiving the estrone during this period showed shrinkages in comb measurements of 11, 8, and 8 mm., with an average regression of 9 mm. The combs of the uninjected control group during the same period grew 16, 14, and 13 mm., with an average increase of 14.3 mm. The average comb measurements for the entire period are shown in figure 1.

The estrone-injected birds in every case but one responded with an actual shrinkage of the comb while in the uninjected birds the combs continued to grow at the former rate. During the rest period of one week both groups showed comb growth indicating that the shrinkage due to estrone is a temporary effect.

After the completion of the first experiments we repeated the study with another group of incomplete castrates using olive oil in place of sesame oil as the injection medium (1/20/38 to 5/5/38). Of the 8 incomplete castrates used, 5 survived.

The control birds received injections daily for 3 weeks of the same amount of pure olive oil as was used in the solution of estrone administered to the experimental series. The dose of estrone was the same as that previously used, 0.5 mg. per day, and one week after the end of the injection period the groups were reversed as in the earlier series. The results recorded graphically in figure 1 were exactly comparable in the two experiments. Blanching of the comb was noted in a few cases in each experiment.

The Effect of Estrone Administration on the Comb Growth of Cockerels

Ten cockerels approximately 3 months old whose combs had been measured weekly for one month were divided into 2 equal groups comparable in initial comb size and rate of comb growth. Six of these birds, 3 in each group, were alive at the end of the experiment (3/15/38 to 5/25/38). Group 1 received daily injections of 0.5 mg. of estrone in 1 cc. of olive oil for 2 weeks. Group 2 received olive oil alone. After 2 weeks of injections both sets were uninjected for one week and as in the experiments with the incomplete castrates the groups were then reversed. Group 2 which formerly

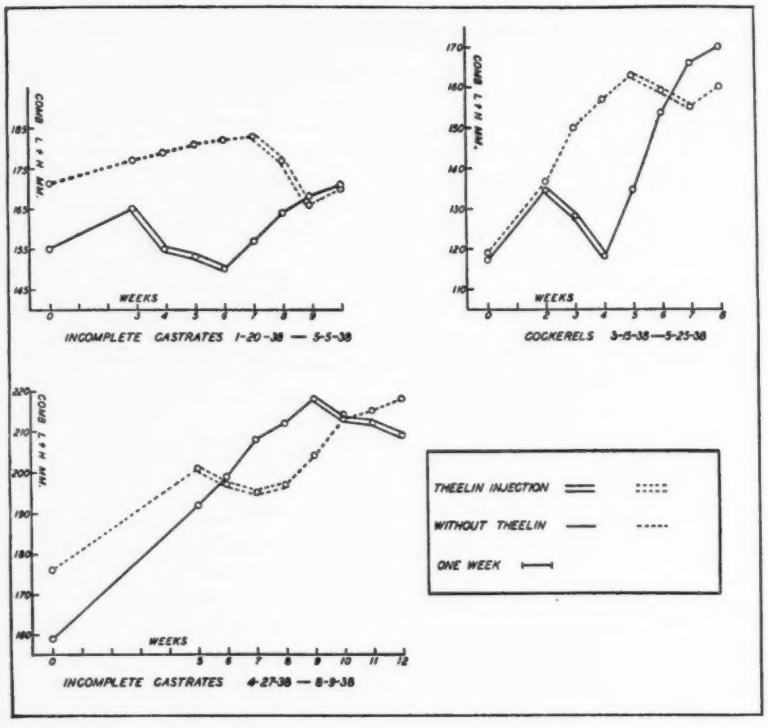


Fig. 1

served as control was given the estrone in olive oil for 2 weeks and Group 1 was given the oil alone. Measurements were continued for an additional week to detect any delayed effect on comb growth. The results (fig. 1) clearly show that estrone injections caused a marked shrinkage in the combs of cockerels with an immediate resumption of growth when injections ceased, precisely as we observed with the incomplete castrates. We likewise noted a distinct blanching of the comb in some but not all of the birds receiving estrone.

After the completion of these experiments, Regnier (4) published an ac-

count of similar experiments on normal cockerels. He determined the effect of estradiol benzoate on cockerels one month, 5 months, and 1½ to 2 years of age. Weekly injections of 0.5 mg. of estradiol benzoate in oil in the month-old cockerels caused a retardation of the growth of the comb. In the 5-month-old cockerels the injection of 1.0 mg. per week was followed by a regression of the comb although comb growth was resumed after a month in spite of continued injections. In the adult animals, 2.0 mg. per week produced an immediate regression which continued for a month after which the comb resumed growth in spite of continued treatment. Growth was resumed more rapidly if a smaller dose was substituted and later injections of a larger dose produced a new regression, also temporary. Our findings confirm those of Regnier to some extent although we were unable to detect the general checking of body growth which he observed.

In order to check this point and to study the effect of estrone on body development we injected 0.5 mg. of estrone per day in oil intramuscularly into 1, 3 and 5 week old cockerels for one or two weeks (8/9/38 to 10/4/38). The results of 3 experiments are summarized in table 1. We found no evidence of any effect on growth, as shown by the body weights, nor any evidences of toxicity from these high doses. In all three

TABLE 1. THE EFFECT OF ESTRONE ON YOUNG COCKERELS

	No.	First weight	Final weight	Comb weight / Body weight	Testis weight / Body weight
A. Week-old cockerels—0.5 mg. estrone per day for one week					
Controls	8	64 gm.	100 gm.	0.072%	0.029%
Estrone-treated	8	64 gm.	98 gm.	0.046%	0.023%
B. Three-week-old cockerels—0.5 mg. estrone per day for one week.					
Controls	13	219 gm.	256 gm.	0.32%	0.035%
Estrone-treated	19	206 gm.	247 gm.	0.12%	0.024%
C. Five-week-old cockerels—0.5 mgm. estrone per day for two weeks.					
Controls	13	337 gm.	496 gm.	0.45%	0.029%
Estrone-treated	10	337 gm.	502 gm.	0.15%	0.020%

experiments the combs of the estrone-treated animals are definitely smaller than those of the controls. There is also a slight but consistent effect on the testis, the estrone-treated birds having smaller testes in all 3 trials. The combs and testes were sectioned and no evidence of changes in the estrone-treated combs or testes were found except in the combs of the week-old cockerels treated with estrone for one week. The walls of the arterioles of the combs in this set were enlarged and stained more deeply than the control combs.

Morató-Manaro, Albrieux, and Buno (5) have also reported similar findings with estradiol benzoate (progynon-B) rubbed into the combs of cockerels. They report a 24.5% reduction in the size of the comb with the application of 43,750 I.U. in 7 days and a parallel atrophy of the testes. Progesterone produced no such effect on the comb or testes.

The Effect of Estrone Administration on the Comb Growth of Capons

The inhibition of comb growth observed in incomplete castrates and in cockerels under the influence of estrone may be explained by a mechanism similar to that presumably operating in the mammal. If, then, the results are due entirely to a disturbance in the pituitary-gonad relationship as postulated by Moore and Price for the mammal, it follows that estrone in the same dosage should be without effect in the capon when androgenic substances are administered, due to the absence of the gonads.

We compared the comb-growth response of groups of capons receiving androsterone alone, estrone alone, and mixtures of androsterone and estrone (5/2/38). Since Gallagher and Koch (6) have shown that the comb-growth response to androgens is proportional to the initial comb length, the capons used were matched before the injections were started so that the average initial comb-length for each group of 20 capons was the same. Intramuscular injections of the various substances dissolved in olive oil were made daily for 5 days and the combs measured on the first and sixth days according to the method of these authors.

Group 1 was injected with 0.1 mg. of androsterone in 1 cc. of olive oil per day. Group 2 received 0.5 mg. of estrone in 1 cc. of oil per day. These solutions were prepared by adding an alcohol solution of the substance to the olive oil and boiling off the alcohol under diminished pressure. Group 3 received both hormones. In preparing this solution, alcohol solutions of estrone and androsterone were added to the olive oil and the alcohol boiled off so that each cc. of olive oil contained 0.1 mg. of androsterone and 0.5 mg. of estrone. Group 4 received 0.1 mg. of androsterone and 0.1 mg. of estrone per cc. of olive oil. In Group 5 the procedure was modified. Five-hundredths (0.05) cc. of a 60% alcoholic solution which contained 0.01 mg. of estrone was applied directly to the surface of the comb daily for 5 days using a tuberculin syringe with a 27-gauge needle.[4] At the same time 0.1 mg. of androsterone in 1 cc. of olive oil was injected intramuscularly each day as in the previous groups. The comb growths of the 5 groups, representing in all 100 capons, are given in table 2.

TABLE 2. THE EFFECT OF ESTRONE AND ANDROSTERONE ON COMB-GROWTH IN CAPONS

Group	No. of capons	Substances administered per cc. per day	Comb growth	Relative activity[1]
			mm.	I.U.
1	20	0.1 mg. androsterone	4.1	1.00
2	20	0.5 mg. estrone	0	0
3	20	0.1 mg. androsterone and 0.5 mg. estrone	1.8	0.27
4	20	0.1 mg. androsterone and 0.1 mg. estrone	3.0	0.60
5	20	0.1 mg. androsterone injected and 0.01 mg. estrone in 0.05 cc. 60% alcohol applied to comb surface	2.8	0.54

[1] Relative activity in international units determined from comb growths by characteristic curve of Gallagher and Koch (6).

The comb growth obtained with androsterone alone in the dosage we have used represents exactly 1.0 I.U., since this is by definition the growth

[4] Control tests showed that 60% alcohol applied to the comb has no effect on the comb-growth response to injected androsterone.

produced with 0.1 mg. androsterone, and this group therefore serves as the standard to which all the other experimental groups are referred. The values in international units are then calculated for the other groups by means of the characteristic curve established by Gallagher and Koch (6).

These results definitely show that the injection of 0.5 mg. of estrone per day for a 5-day period is without effect on the size of the capon comb. The international unit of androsterone caused an average increase of 4.2 mm. When, however, this same amount of androsterone (0.1 mg.) is administered simultaneously with 0.5 mg. of estrone, the average comb-growth response is 1.8 mm. (0.27 I.U.) which represents a decrease of 73%. When a smaller amount of estrone, 0.1 mg., is administered simultaneously with 1 I.U. of androsterone, the comb-growth response is still less than that obtained with androsterone alone but is significantly greater than that with the larger amount of estrone. The average growth in this group is 3.0 mm. (0.60 I.U.) a decrease of 40%. The last group where a still smaller amount of estrone (0.01 mg.) is applied to the surface of the comb indicates that the comb-growth response under the influence of 1 I.U. of androsterone is practically the same as that obtained when ten times the quantity of estrone is injected intramuscularly. The average growth in this group is 2.8 mm. (0.54 I.U.), a decrease of 46% from that observed with androsterone alone.

Mühlbock (7) has studied this reaction in some detail although we were not aware of his work at the time our experiments were carried out. Gley and Delor (8) have also published a note on the inhibiting effect of estradiol benzoate on the comb-growth response to testosterone propionate which they observed in one capon. Mühlbock's findings are similar to ours but are more extensive in regard to some points. He found that when 0.4γ (0.0004 mg.) of testosterone per day was smeared on the comb of the capon for 4 days and varying amounts of estrogens were injected each day, an interference with the comb growth was produced, ranging from complete inhibition with 0.5 mg. per day of estrone, estradiol, and progesterone, to no effect when smaller amounts of these substances or 0.5 mg. doses of estriol, equilenin, or pregnenolone were injected. The specific action of certain compounds is of great importance in interpreting the mechanism of the interference and will be dealt with in the discussion. The comb-growth response was also interfered with when 0.1 mg. of androsterone per day for 4 days was injected into capons and the estrogens injected in parallel. Under these conditions, 0.02 mg. to 1.0 mg. of estrone per day caused only slight interference, 5.0 mg. of estradiol benzoate caused complete inhibition, and 0.2 mg. of progesterone produced some interference. These last experiments are more comparable to ours although Mühlbock injected for only 4 days instead of 5 and expressed comb growths in per cent increase in comb area rather than in millimeters increase in length and height so that a direct comparison of his results with ours is impossible.

DISCUSSION

Our experiments clearly demonstrate that estrone lessens the comb-growth response and that this effect is independent of the source of andro-

gen. The similarity in result whether the hormone is supplied by the gonad, as in the case of cockerels and incomplete castrates, or is introduced by intramuscular injection or local application as in the experiments on capons, is quite striking.

It would appear then that the testis is not the necessary agent in the inhibition of comb growth by estrone except as it serves as a source of male hormone, for estrone likewise suppresses the response to androsterone in the capon. Since a similar effect can be secured in the absence of the testis one is forced to the conclusion that the usual pituitary-gonad relationship cannot be the only effective mechanism. There is no doubt that the comb-inhibition is similar in cockerels and capons but a possible quantitative difference is indicated by the relative reaction of the two. The dose of estrone used in the experiment on cockerels caused an actual regression in the size of the combs of birds growing at a rate of 5 to 8 mm. during any 5-day interval. The capons were given an amount of androsterone which produced an average increase of 4 mm. in the control group. It might be expected that the same quantity of estrone would completely eliminate the comb response in the capon experiment. Since, however, a definite although diminished growth occurred, it could be argued that in the cockerel we were dealing with a more complex reaction than that existing in the capon. It should, however, be borne in mind that the experiments themselves are not strictly comparable since the cock's comb and a capon's comb are in an entirely different physiological condition.

The data of Mühlbock as to the specific nature of the ability to produce inhibition on the capon comb is of interest in attempting to explain the action. The ability of estrone and estradiol to interfere with the comb-growth response to testosterone while an equal dose of equilenin or pregnenolone is without effect appears to rule out an interference by a non-specific substance. As Mühlbock points out, the estrogenic substances that are the most active in the female organism are likewise the most effective in the inhibition of the action of testosterone. When we applied 0.01 mg. of estrone per day directly to the comb of the capon and injected androsterone in parallel the inhibition was as marked as when 10 times this amount of estrone was injected. This difference is indicative of a local action on the comb and in this connection the blanched appearance of the comb in cockerels injected with estrone takes on added significance. Such blanching immediately suggests a decreased blood supply to the comb. A possible mechanism by which estrone could bring about an interference with the blood supply to the growing comb is indicated by its action in causing smooth-muscle hypertrophy, a property of estrone manifest in males as well as in the familiar action on the uterine musculature. A similar action of estrone on the smooth muscle in the walls of the arterioles of the comb would diminish the blood supply and thereby diminish growth. Such a mechanism would account for the marked effect in cockerels where the comb is highly vascularized and would similarly explain the lessened response in capons. There is as yet no direct evidence for or against this mechanism. In the week-old cockerels injected with estrone for one week there were strong indications of such an effect on the arterioles

of the combs, these arterioles having enlarged and deep-staining walls, but this condition was not found in the combs of the cockerels 3 and 5 weeks of age. In the cases reported by Juhn, Gustavson, and Gallagher (3) where a slight shrinkage of the comb was noted, it was associated with a disappearance of fat from the central core of the comb. No other histological changes were reported. The possibility of more effective absorption of estrone when it is applied to the surface of the comb instead of injected may of course be a factor.

In attempting to elucidate the mechanism involved in the effect of estrone on the comb response of cockerels and capons, the following possibilities must be considered. A local action of the estrone on the comb is indicated by the blanching of the comb and the increased effectiveness of locally applied estrone. Such a local action does not preclude the possibility of a pituitary effect operating such as is postulated for mammals. This may be the case with cockerels where the estrone injected inhibits the gonadotropic output of the pituitary thus decreasing the male hormone output of the testis and resulting in an actual shrinking of the comb. The fact that the comb-growth inhibition is demonstrable to a qualitatively similar but quantitatively different extent in the capon, where the pituitary-gonad relationship is ruled out by the absence of the testis, indicates that such a relationship cannot be the sole factor. We believe that at least two mechanisms, estrone acting directly on the comb, and indirectly by inhibiting the pituitary, are involved. The direct action on the comb appears to be the principal factor. The significance of the apparent correlation between the estrogenic activity of a substance and its ability to inhibit comb growth is not yet known. The rôle of the comb in the sexual physiology of the cockerel is as yet incompletely disclosed. Although it is usually considered to be a passive organ, merely responding to the androgen secreted by the testis and exerting no reciprocal action, recent investigations by Zawadowsky and others (9, 10, 11, 12) have indicated that it may play a more important part than previously suspected. The physiological meaning of the ability of estrogens to inhibit this organ must await further developments in our knowledge of its function.

An important corollary to our experiments appears in the assay for androgenic substances by the comb method. In all but the most extraordinary cases the concentration of estrone or other estrogens in crude extracts would be far too small to produce a significant difference in comb growth when the injection procedure in capons (6) is followed. When, however, the extract is applied directly to the comb—as in the inunction method of Fussgänger (13)—the advisability of fairly complete removal of estrogenic material is indicated particularly when large quantities of estrogens are suspected. This may be of special significance in assaying urine extracts in various pathological conditions where the amount of estrogenic substance is unknown.

SUMMARY

The intramuscular injection of 0.5 mg. of estrone daily for 2 or 3 weeks into cockerels and incompletely castrated cockerels produced a temporary shrinkage in the comb with resumed growth as soon as the injections were

discontinued. In cockerels 1 to 5 weeks of age treated with this dose of estrone for 1 or 2 weeks the combs and testes were smaller than those of the controls. The simultaneous administration of estrone and androsterone to capons produced a smaller comb growth than androsterone alone. Estrone applied to the surface of the comb of the capon was 10 times as effective as intramuscularly injected estrone in inhibiting comb-growth response to injected androsterone. The estrone apparently acts directly on the comb and indirectly through the pituitary. This inhibiting action of estrogens emphasizes the necessity of separating urine extracts into androgenic and estrogenic fractions if the androgens are to be applied directly to the comb or in pathological cases where high concentrations of estrogens may be excreted.

REFERENCES

1. Moore, C. R., and D. Price: *Am. J. Anat.* 50: 13. 1932.
2. Juhn, M., F. D'Amour and E. B. Womack: *Am. J. Physiol.* 95: 641. 1930.
3. Juhn, M., R. G. Gustavson and T. F. Gallagher: *J. Exper. Zool.* 64: 133. 1932.
4. Regnier, V.: *Compt. rend. soc. de Biol.* 127: 519. 1938.
5. Morató-Manaro, J., A. S. Albrieux and W. Buno: *Klin. Wchnschr.* 17: 784. 1938.
6. Gallagher, T. F., and F. C. Koch: *J. Pharm. & Exper. Therap.* 55: 97. 1935.
7. Mühlbock, O.: *Acta brev. Neerl.* 8: 2/3, 50. 1938.
8. Gley, P., and J. Delor: *Compt. rend. soc. de biol.* 125: 813. 1937.
9. Zawadowsky, M. M.: *Bull. d. Biol. e. Med. exper.* 1: 196. 1936.
10. Buckner, G. D., W. M. Insko and J. H. Martin: *Poultry Sci.* 71: 365. 1934.
11. Marlowe, H. W.: Personal communication.
12. Hoskins, W. H., and F. C. Koch: Unpublished.
13. Fussgänger, R.: *Med. u. Chem.* (I. G. Farbenindustrie) Leverkusen: 194. 1934.

HYDROGEN ION CONCENTRATION CHANGES IN THE VAGINAL FLUID OF THE RAT DURING AN ESTROUS CYCLE

JACOB S. BEILLY

From the Gynecological Endocrine Laboratory and Clinics, Department of Obstetrics and Gynecology, Beth-El Hospital

BROOKLYN, NEW YORK

THE FUNDAMENTAL observation on the cyclical changes in the vaginal tube of the guinea pig by Stockard and Papanicoloau, later, in rodents, by Allen, Long and Evans, remains a great contribution to our present-day knowledge. Having served as an impetus to further research, it is employed as a basis in clinical investigation and biological assay.

The implication that the contents of the vaginal fluid reflects the cyclical changes of the genital tract was founded upon a changing cytology. This is comparable to the changes occurring in the vaginal tube. In the adult female rodent, rat or mouse, the various phases can be pointed out. However, the cyclical change, as the word implies, is continuous and these overrun into one another, in the relatively short space of time of a cycle.

Our clinical observations[1] on the changing pH of the vaginal fluid in women, has implied the concept that the pH of the vaginal fluid in the rodent such as the rat, will bear close correlation with the cyclical changes as reflected by cytology of its content.

METHOD AND DETERMINATION

Thirty animals in several groups of adult female, and castrate rats, weighing 130 to 160 gm., hooded and albino species, were used.

Hydrogen ion determination was carried out in glass-cells, by the spot method (LaMotte).

Histological criteria taken conformed closely to those commonly referred to as definite phases in a cycle. Our experience led us to fix the histological criteria from a point in the cycle of very short duration (8 to 12 hours) best described as 'epithelization.' Estrus can be identified as that immediately following it. These criteria are demonstrated in figure 1, 2, 3.

The combined method of smear demonstration and hydrogen ion determination were carried on during various periods; before and after castration, before and after administration of estrogens,[2] by injecting 8 international units of progynon-B, subcutaneously. The pH of the vaginal fluid was determined, and vaginal smears were made, simultaneously, and recorded separately. Comparative studies followed and table 1 contains the observa-

[1] The clinical implications will be dealt with in a subsequent paper.

[2] Progynon-B was generously supplied for this study through the kindness of Dr. W. Stoner of Schering Corporation, Bloomfield, New Jersey.

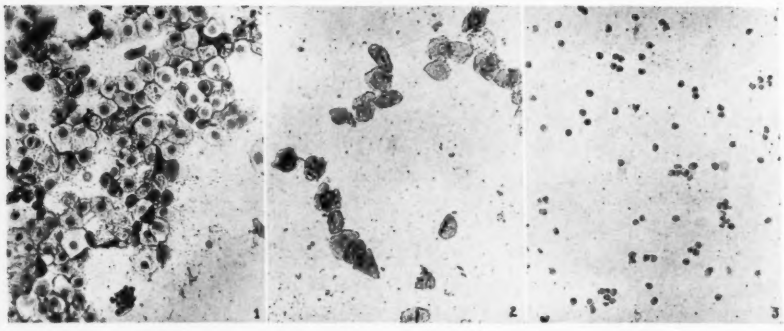


Fig. 1. *Animal No.* 5 Epithelization—E—×124 (Proestrus) pH 6.4. Fig. 2. *Animal No.* 5 Cornification—Es—×124 (Estrus) pH 4.6. Fig. 3. *Animal No.* 8 Leucocytic—L—×124 (Anestrus) pH 7.4.

tions which represent the histological criteria and comparable pH determinations. Graphs I and II (fig. 4) represent composite curves for the normal and castrate group of animals, respectively.

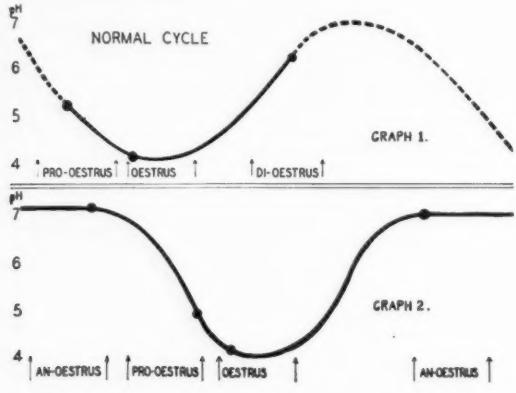


Fig. 4

SUMMARY

There is a changing pH in the vaginal fluid. It is cyclical in occurrence. The acid phase is associated with 'epithelization' and estrus; the alkaline phase with leucocytic or di-estrous stage. When the leucocytes predominate, the pH of the fluid changes toward alkalinity.

It seems as if the variation in the pH is associated with the changing cytology in the vaginal fluid. Further studies are under way to determine whether, in this association, the fluid element of the vaginal contents plays a part.

The pH of vaginal fluid does not materially vary in the castrate. A changing pH can be induced upon the return of cycle, after administration of estro-

gens. These variations in the pH run a course similar to that found in the estrus cycle of adult female, non-castrate; and similarly, it seems to be associated with the changing cytology of the fluid contents.

TABLE 1. pH OF THE VAGINAL FLUID IN THE RAT IN THE NORMAL ADULT CASTRATE AND AFTER ADMINISTRATION OF ESTROGENS

Normal Vaginal Cycle

Rat No.	Proestrus		Estrus		Diestrus	
	Micros.	pH	Micros.	pH	Micros.	pH
1	E	5.4	Es.	4.4	L(E)	7.0
2	E	4.8	Es.	4.4	LE	6.8
3	E	6.0	Es.	5.6	LE	5.6
4	E	4.8	Es.	4.4	LE	5.6
5	E	5.4	Es.	4.6	LE	6.8
6	E	4.4	Es.	4.6	L[1]E	6.2

Castrated 8 I.U. Progynon-B

Rat No.	Anestrus[2]		Proestrus		Estrus		Anestrus	
	Micros.	pH	Micros.	pH	Micros.	pH	Micros.	pH
7	L	7.4	LE	5.0	Es.	5.0	L(E)	7.0
8	L	7.4	E	4.4	Es.	4.6	L	7.2
9	L	7.2	E(C)	4.6	Es.	4.8	L	7.2
10	L	7.4	E[1]Es	6.0	Es.	4.8	L(E)	7.4
11	L	7.2	E(C)	4.4	Es	4.6	LE	5.2
12	L	7.4	E	4.4	Es.	4.8	L	7.0
13	L	7.2	LE(C)	4.6	Es	4.4	L	6.8
14	L(E)	6.8	LE[1]	4.4	Es.	4.6	L	7.0
15	L	6.8	E[1]C	4.4	Es.	4.4	L	7.0

E —Epithelization.
Es.—Estrus.
C —Cornification
L —Leucocytes.
( ) —Few.
[1] Many.
[2] Conditions before injection.

The pH determination of the vaginal fluid will identify the phase of cyclical change in the genital tract. It has its practical value when applied in the breeding of rodents.

I am deeply indebted to Dr. Raphael Kurzrok of the Presbyterian Medical Center, New York City for his encouragement and stimulating advice; to Dr. Mendel Jacobi, director of laboratories, for his cooperation and helpful suggestions; and to Drs. Koplowitz and Oginz for their broad vision in creating a departmental laboratory, which made it possible to undertake this investigation. I highly appreciate the technical assistance rendered by Mr. Alvin Dubin.

PHYSIOLOGICAL STUDIES IN INSULIN TREATMENT OF ACUTE SCHIZOPHRENIA

THE CHOLINE ESTERASE ACTIVITY OF THE BLOOD SERUM

LOWELL O. RANDALL AND E. M. JELLINEK

From the Research Service of the Worcester State Hospital and the Memorial Foundation for Neuro-Endocrine Research

WORCESTER, MASSACHUSETTS

THE RÔLE of acetyl choline as a chemical mediator in the autonomic nervous system and, perhaps, the central nervous system, on the one hand, and the rôle of choline esterase in splitting acetyl choline, on the other hand, have given rise to considerable speculation. The question whether or not the choline esterase activity of the blood serum excercises any regulating function on autonomic activity is still undecided. Ginsberg, Kohn and Necheles (1) reviewed the status of investigations on these subjects previous to 1937. While many of the investigators quoted by these authors are inclined to see a definite relation between the autonomic nervous system and choline esterase activity, the investigations of Ginsberg and his collaborators led to the conclusion that " . . . the esterase level of the blood is a rather immobile, unresponsive bulwark against an excess of acetyl choline in the blood." Other investigations published at about the same time or later than the paper mentioned above are still somewhat conflicting. Milhorat (2) found no differentiation of the choline esterase level of the blood serum in various physical diseases except a lowering in patients with marked debilitation. On the other hand, Jones and Tod (3) succeeded in lowering the choline esterase level with eserine, as well as with prostigmin, and raising the level with the administration of carbaminoylcholine chloride. Later, Tod and Jones (4) reported great differences in the choline esterase level of anxiety and stuporous types of mental disorders, giving evidence of a direct relationship between the autonomic activity and the choline esterase level.

Since insulin coma provokes a heightened activity of the sympathetic nervous system, we wished to investigate whether or not a prolonged administration of insulin in coma dosages would cause a perceptible change in the choline esterase activity of the blood serum, and whether or not there was an association between such changes and the clinical amelioration frequently found in the insulin treatment of schizophrenic patients.

The choline esterase of the blood serum of the 22 patients described in the introductory paper was determined twice in the two weeks preceding insulin treatment and twice in the two weeks after stopping treatment. The gasometric method of Ammon (5) was used. The volume of carbon dioxide

liberated from a bicarbonate-Ringer's solution by the acid formed from the hydrolysis of acetyl choline substrate was measured in the Warburg apparatus. After equilibration for 20 minutes the reaction between serum and acetyl choline was allowed to continue for 60 minutes at 37° C. A blank was run to determine the spontaneous hydrolysis. The activity of the enzyme was expressed as cu. mm. of CO_2 evolved per hour per cc. of serum.

RESULTS AND DISCUSSION

Before insulin administration started the 22 schizophrenic patients investigated had an average choline esterase level of 71.0 cu. mm. of CO_2. The minimum value was 40.8 and the maximum was 96.0 cu. mm. In 20 normal controls we found a range from 35.5 to 135.8, with a mean of 76.4. The difference between patients and normal controls relative to the mean values was not significant. Tod and Jones (4) found in 24 schizophrenics a mean of 76 and in 30 normal controls a mean of 83.5. These absolute values differ slightly from ours, but the difference between the normal and the patient values is approximately the same in our series as in the series of Tod and Jones. In view of the extraordinary variation among normal controls, one could hardly speak about a depressed level of choline esterase in subjects of acute schizophrenia.

In the following table the means and intra-individual standard deviations are given separately for the 'recovered' and 'not-recovered' patients before and after treatment, and also those for normal controls. The actual observations are shown in frequency distributions on the accompanying graph.

MEANS AND INTRA-INDIVIDUAL STANDARD DEVIATIONS OF CHOLINE ESTERASE (IN TERMS OF CU. MM. CO_2)

	29 Normal controls	Patients			
		10 'Recovered'		12 'Not-recovered'	
		Before medication	After medication	Before medication	After medication
Mean	76.4	72.7	86.5	69.6	80.9
Intra-individual standard deviation	3.46	3.87	1.61	4.12	5.10
		Significance of difference between means	$t=5.97$ $P<.01$	Significance of difference between means	$t=4.25$ $P<.01$

The 'recovered' and 'not-recovered' patients did not differ initially from each other in their means. After medication the former group showed a mean rise of 20% and the latter group of 17%. Both these increases are statistically significant as can be seen from the t criteria in the table. The post-medicational means are both above the normal control value. There is indication that insulin has brought about an elevation of the choline esterase level above normal requirements, in that in the course of the two weeks following the medication the choline esterase level tended to drop off in both types of patients. In the first week following medication the mean for the 'recovered'

patients was 88.4 and in the second week 82.0. The corresponding values for the 'not-recovered' patients were 84.6 and 79.9, respectively.

The concomitance of a rise in choline esterase activity with a stimulation of the sympathetic nervous system following insulin treatment may be interpreted as a compensatory response. The choline esterase activity of serum is remarkably constant within individuals under ordinary conditions. Only under extreme conditions, such as prolonged insulin administration, is the reserve inadequate resulting in an increased amount of activity of the enzyme in serum and at end organs.

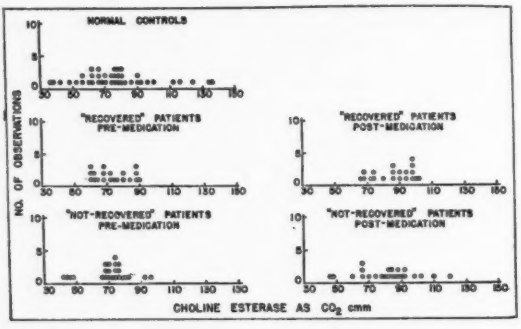


Fig. 1. Frequency distributions of the choline esterase of the blood serum.

While the average levels of choline esterase were raised by insulin irrespective of clinical outcome the intra-individual standard deviations (see table) show that insulin action was nevertheless of an essentially different nature in 'recovered' and 'not-recovered' patients. In the former the intra-individual standard deviation decreased after medication significantly from 3.87 to 1.61 (ratio of squared values: 5.76; $P<.01$)—i.e., significantly far below the normal control value. This may indicate an overstabilization of the function which may or may not have its disadvantages. In contrast to this the 'not-recovered' patients increased in intra-individual variation from 4.12 to 5.10. While this increase is not statistically significant, the post-medicational intra-individual standard deviation of the 'not-recovered' patients is probably significantly greater than that of normal controls (ratio of squared values: 2.17; $P=.05$). In the 'not-recovered' patients the level of choline esterase was raised only at the expense of increased instability of the function. The action of insulin on the lipids, too, was to decrease the intra-individual variation in the 'recovered' patients and to increase it in the 'not-recovered' patients, while both patient groups showed the same increases in the average levels (see preceding paper). The difference between 'recovered' and 'not-recovered' patients is reflected to a greater degree in the effect of insulin on the stability of the blood constituents than in its action on their average levels.

In conclusion it may be pointed out that neither the initial level of choline esterase nor the magnitude of the insulin dosage showed any correla-

tion with the amount of change from premedicational to post-medicational choline esterase values.

SUMMARY

The choline esterase activity of the blood serum of 22 schizophrenic patients in the acute stage was determined by the gasometric method of Ammon twice in the 2 weeks preceding and twice in the 2 weeks following insulin treatment.

The choline esterase level of the patients was initially somewhat lower than that of the normal controls. This difference, however, by statistical test was not significant.

Following insulin treatment the choline esterase level increased significantly irrespective of whether the patients recovered or not.

In the 'recovered' patients the intra-individual variation was greatly lessened following treatment, the function becoming stabilized while in the 'not-recovered' patients the intra-individual variation was significantly increased; i.e., the function became highly unstable.

In both types of patients the choline esterase level tended in the second week following medication to decrease towards the initial level.

REFERENCES

1. Ginsberg, R., R. Kohn and H. Necheles: *Am. J. Dig. Dis. & Nutrition.* 4: 154. 1937.
2. Milhorat, A. T.: *J. Clin. Investigation* 17: 9. 1938.
3. Jones, M. S., and H. Tod: *J. Ment. Sci.* 83: 202. 1937.
4. Tod, H., and M. S. Jones: *Quart. J. Med.* 6: 1. 1937.
5. Ammon, R.: *Arch. f. d. Ges. Physiol.* 233: 486. 1933.

PHYSIOLOGICAL STUDIES IN INSULIN TREATMENT OF ACUTE SCHIZOPHRENIA

THE BLOOD MINERALS

J. M. LOONEY, E. M. JELLINEK AND CORA G. DYER
From the Memorial Foundation for Neuro-Endocrine Research and the Research Service of the Worcester State Hospital
WORCESTER, MASSACHUSETTS

THE IMPORTANCE of phosphorus to the metabolic functioning of the body has long been recognized. Various compounds containing it are found in all the tissues of the body. These compounds are intimately involved in the intermediate metabolism of glucose in several types of protid synthesis and in the metabolism of lipids, nucleic acid and calcium. They are also involved in muscular contraction and are essential for the functioning of the central nervous system. Hence, any investigation of the effects of insulin would be incomplete without a study of the changes in phosphorus.

Recently Verzár and Laszt (1) have postulated that the symptoms of adrenal insufficiency are due to an inhibition of phosphorylation of phosphatids. Pijoan and Oberg (2) and Verzár and Laszt (3) have also suggested that the adrenals act to convert flavin to flavin phosphate. An indication of adrenal insufficiency may be found in part in the low blood pressure of schizophrenic patients. The fact that the blood pressure reaction to glycerol extracts of adrenal cortex is much greater in these patients than in normal controls further strengthens this argument (4).

Potassium decreases during insulin coma. The exact mechanism of this fall is not known, but it may be surmized that the potassium is used up in part in forming salts of the hexose-phosphate complex. The interdependence of calcium and phosphorus led us to include the former as a variable in our investigation.

METHODS

Inorganic phosphorus, potassium and calcium of the blood were determined 4 times on each patient in the 2 weeks preceding and 4 times in the 2 weeks following insulin therapy. Phosphorus was determined by the method of Fiske and Subarrow on trichloroacetic acid filtrates of blood serum (5). Calcium was determined by the Clark-Collip modification of the Kramer-Tisdall method (6). Potassium was determined by precipitation with silver cobaltinitrite as suggested by Breh and Gaebler (7), and the potassium in the precipitate determined colorimetrically by the diazo method of Briggs (8).

RESULTS AND DISCUSSION

Neither the mean levels nor the variations of blood calcium and potassium

TABLE 1. MEAN VALUES OF BLOOD CALCIUM AND POTASSIUM

	27 Normal controls	Patients			
		10 'Recovered'		12 'Not-recovered'	
		Before medication	After medication	Before medication	After medication
	Mean values in mg. %				
Calcium	10.5	10.5	10.4	10.5	10.5
Potassium	20.0	21.9	21.9	21.3	21.5

showed changes following prolonged administration of insulin. There were also no differences found between 'recovered' and 'not-recovered' patients, nor between patients and normal controls. The mean values of these constituents are given in table 1.

The blood phosphorus, on the other hand, showed changes of great interest. These changes were limited to the mean levels only, and the measures of variation, therefore, will not be discussed. The shifts of the mean values which followed insulin medication gain in significance through the fact that these patients suffering from acute schizophrenia differed initially in their phosphorus levels from normal controls as well as from patients in the sub-acute stage previously tested here (table 2).

For such a constituent as phosphruos, a difference of 0.37 mg./100 cc. is of physiological importance. The differences are also statistically significant, as can be seen from the values in table 2. It would thus seem that there is an elevation of the blood phosphorus above normal in acute schizophrenia, but that there is a tendency towards restoration to the normal level in the course of the schizophrenic process as is indicated by the mean value in the patients in later stages of the psychosis.

The accompanying graph shows the phosphorus values of the 'recovered' patients shifted after medication to a much lower level. The 'not-recovered' patients, too, showed a drop, but as can be seen from the frequency distributions, this was not nearly as uniform as in the 'recovered' patients. The mean values and significance criteria of differences between means are tabulated (table 3).

TABLE 2. COMPARISON OF MEAN PHOSPHORUS VALUES IN THE ACUTE AND SUB-ACUTE STAGES OF SCHIZOPHRENIA AND IN NORMAL CONTROLS

	Mean (mg./100 cc.)		Significance of mean differences	
			t	P
(1) Acute schizophrenia (22 patients of the insulin series)	3.95	Acute schiz. *vs.* sub-acute schiz.	2.5	$<.02>.01$
(2) Sub-acute schizophrenia (28 patients)	3.63	Acute schiz. *vs.* normal controls	3.6	$<.01$
(3) 27 Normal controls	3.58			

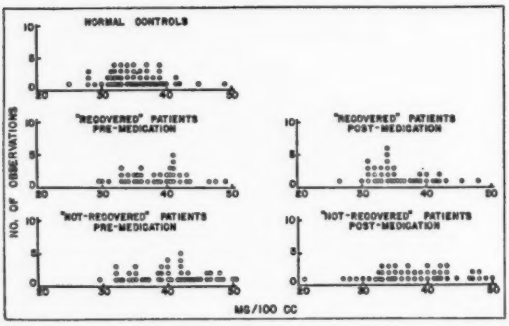


Fig. 1. Frequency distributions of inorganic phosphorus of the blood.

The mean change for the 'recovered' as well as for the 'not-recovered' patients was 0.25 mg./100 cc. This, however, brought the 'recovered' patients exactly to the normal level, while the 'not-recovered' patients were still significantly 18 mg. above the normal mean. From table 3 it is seen that the change in mean levels was highly significant for the 'recovered' patients, but was not significant for the 'not-recovered' patients. Looking at the graphs one can understand why a drop of 0.25 mg. should be significant in one instance but not significant in the other. This differentiation through mean

Table 3. Mean phosphorus values before and after medication and criteria of significance of differences between means

	10 'Recovered' pts.	12 'Not-recovered' pts.
	Mean phosphorus (mg./100 cc.)	Mean phosphorus (mg./100 cc.)
Before medication	3.84	4.05
After medication	3.59	3.81
Significance of difference of means	$t=3.6$ $P< .01$	$t=1.7$ $P> .05$

levels is of great interest, since it occurred in only two other variables, namely, systolic blood pressure and pulse rate. In all other variables the differentiation was limited to the intra-individual variation.

Thus, it would appear that the elevation of the blood phosphorus level in acute schizophrenia is an important feature, and that its normalization by insulin is probably a significant element in the mechanism of remission. The decrease of inorganic phosphorus in the 'recovered' patients may be due to its better utilization in the formation of phosphorylated compounds.

SUMMARY

Potassium, calcium and phosphorus of the blood were determined on 22 schizophrenic patients 4 times in the 2 weeks preceding and 4 times in the 2 weeks following insulin treatment. Potassium and calcium showed no changes following medication. The mean level of the inorganic phosphorus

of the blood was initially significantly higher than in normal controls. The phosphorus level of the 10 patients who recovered dropped significantly after insulin treatment to the normal level. The 'not-recovered' patients, however, showed an insignificant decrease only.

REFERENCES

1. Verzár, F., and L. Laszt: *Biochem. Ztschr.* 285: 356. 1935; 288: 356. 1936.
2. Pijoan, M., and S. A. Oberg: *Proc. Soc. Exper. Biol. & Med.* 36: 187. 1937.
3. Verzár, F., and L. Laszt: *Arch. f. d. ges. Physiol.* 237: 476. 1936.
4. Freeman, H., and R. G. Hoskins: *Endocrinology* 18: 576. 1934.
5. Fiske, C. H., and Y. Subarrow: *J. Biol. Chem.* 66: 375. 1921.
6. Clark, E. P., and J. B. Collip: *J. Biol. Chem.* 63: 461. 1925.
7. Breh, F., and O. H. Gaebler: *J. Biol. Chem.* 87: 81. 1930.
8. Briggs, A. P.: *J. Biol. Chem.* 57: 351. 1923.

CHANGES IN GROWTH AND FUNCTION OF THE THYROID AFTER THYROTROPIC STIMULATION

WINDSOR C. CUTTING

From the Department of Pharmacology, Stanford University School of Medicine
SAN FRANCISCO, CALIFORNIA

THE EXPERIMENTAL use of colchicine makes it possible to separate changes of function from changes of growth in thyroid glands stimulated by thyrotropic extracts. Thyroid function is ordinarily estimated by metabolic, and to some extent histological, changes, while growth is determined by the incidence of mitosis. The latter may be made evident by colchicine, which arrests cell division in the metaphase, thus allowing all mitoses to be counted for a considerable period.

This paper reports the use of these methods in a study of the correlation of function and growth of the thyroid at various times after the induction of experimental hyperthyroidism.

METHODS

Female, virgin guinea pigs, weighing about 250 gm. each, were injected daily for periods up to 4 weeks, with from 10 to 25 mg. of a flavianate (1) extract of beef anterior pituitary glands. Simultaneously, with the final injection of this extract, colchicine (or the colchicine derivatives colchicein or oxycolchicine), 0.1 mg., was given in solution subcutaneously. Ten hours later the animals were killed, and the thyroid examined histologically for evidence of hyperplasia, and the presence of increased mitosis. At weekly intervals or, in the shorter experiments more frequently, the metabolic rate was determined with the aid of a device described by Tainter and Rytand (2).

RESULTS

In guinea pigs with experimental hyperthyroidism, the histological evidences of increased thyroid activity, i.e., absorption of colloid and heightening of acinar epithelium, appeared during the first day, and were still present in minor degree at the termination of the experiment when the animals had become refractory. Metabolic changes corresponded, in general, but were slower in appearance, and had usually disappeared by the end of the experiment. Mitotic activity, however, increased sharply on the second day, and had returned to normal at the end of a week. The results obtained are summarized in table 1. Increases in metabolic rate are rated as follows: 0 = normal, + = 1 to 25% increase, ++ = 25 to 50% increase, +++ = over 50% increase. Increases in histological evidence of activity are rated from 0 to ++++, according to criteria previously reported (3). The number of mitoses are indicated as follows: 0 = less than 1 per high power field, + = 1

to 5 per high power field, ++ = 5 to 10 per high power field, and +++ = over 10 per high power field.

TABLE 1. EFFECTS OF INJECTING THYROTROPIC EXTRACTS IN GUINEA PIGS RECEIVING COLCHICINE

	Controls	1 day	2 days	3 days	4 days	5 days	1 wk.	2 wk.	3 wk.	4 wk.
No. of guinea pigs	3	4	2	5	2	2	2	3	3	5
Increase in metabolic rate	−	−	−	±	+	+	++	+++	++	−
Increase in histological activity of thyroid	−	±	+	+++	+++	+++	++++	++++	+++	±
Increase in number of mitoses of thyroid	−	−	+	++±	++±	+	−	−	−	−

DISCUSSION

The evidences for increased function of the thyroid gland in experimental hyperthyroidism in guinea pigs, i.e., increased metabolic rate of the animal and histologically active thyroids run very comparable courses. They appear shortly after the induction of hyperthyroidism, reach a peak in about 2 weeks, and disappear with the onset of refractoriness at about 4 weeks. That they are not paralleled by the number of cell divisions is interesting, and this presumably indicates that, after an early burst of growth, a normal or nearly normal rate of cell proliferation suffices to maintain the hyperactive gland.

CONCLUSIONS

The administration of thyrotropic hormone to guinea pigs causes continued increase of thyroid function as indicated by metabolic and histological changes, until refractoriness is induced.

However, growth of the thyroid gland, as shown by the presence of increased numbers of mitoses, demonstrated by histological examination after the injection of colchicine, is significantly increased only briefly during an early period of the hyperthyroidism.

REFERENCES

1. EVANS, H. M., K. MEYER AND M. E. SIMPSON: *Memoirs Univ. Calif.* 11: 26. 1933.
2. TAINTER, M. L., AND D. A. RYTAND: *Proc. Soc. Exper. Biol. & Med.* 32: 361. 1934.
3. EMERSON, K., J., AND W. C. CUTTING: *Endocrinology* 23: 439. 1938.

SOME EXPERIMENTS ON SALT AND WATER METABOLISM IN DIABETES INSIPIDUS

H. G. SWANN

From the Department of Physiology, University of Chicago
CHICAGO, ILLINOIS

THE CAUSE of the diabetes insipidus (d.i.) following removal of the posterior hypophysis or hypothalamic lesions is still in many points obscure. At the present moment, the theory is widely held that it is a renal phenomenon. In the first place, in some species the operation is followed first by a polyuria and subsequently by a polydipsia (1, 2). Also, by removing the kidneys (or ureteral ligation), Richter (1, 3) has shown that in the rat the polyuria of d.i. is primary to the polydipsia, since this operation abolished the animals' excessive thirst. By employing various other methods in the d.i. cat, Fisher, Ingram and Ranson (2) have come to the same conclusion. The renal defect in d.i. is thought to be due to the lack of secretion of the antidiuretic hormone following the operations (4, 5). In turn, this causes a defective renal tubular resorption of water (6, 7).

On the other hand, the older theory that d.i. is due to some sort of pathological thirst has several points in its favor. In dogs, the polydipsia following hypothalamic lesions may appear before the polyuria does (8, 9, 10) and the same has been reported for man (11). Also Bellows and Van Wagenen (10) found that the water intake of d.i. dogs with esophageal fistulas can be considerably restricted without serious effects. Such dogs continue to 'sham-drink' large quantities of water. Thirst, it is suggested, evidently plays an important part in the etiology of d.i. Bellows (12) has also reported that a nephrectomy of the d.i. dog does not abolish polydipsia as has been reported in the rat.

An experiment that has often been done (2, 8, 9, 10, 13, 14) attempting to demonstrate the primacy of either the polydipsia or the polyuria is that of artificial restriction of the fluid intake of d.i. animals. In spite of the restriction, dogs in the early (or transient) phase of di. continue to excrete excess water (8, 9, 10, 13, 14). This is not clearly true of the cat (2). When restriction of the fluid intake is carried out in the permanent phase of d.i., the animals for a period have a negative water balance, but then this stops, they excrete concentrated urines, and tolerate the restriction for long periods (9, 10). With extreme restriction, however, death may result (2). Such experiments are rather indecisive and so it is not surprising to find them interpreted in opposite ways (2, 10). Fisher, Ingram, and Ranson (2) point out that the antidiuretic hormone of the posterior hypophysis is certainly only one of many controls of renal function and that in the restriction experiments other unknown controls must come into play.

We (15) have recently reported studies of the effect of NaCl on rats with d.i. Saline drinking solutions will cause extreme aggravations of d.i.; often such treatment is followed by the animal drinking 1000 cc. per kg. per day, i.e., a quantity of fluid equal to the animal's weight consumed daily. Such extreme d.i. makes an inter-

esting test object for repetition of the controverted experiments on nephrectomy and fluid restriction in d.i. Confirmation of experiments showing that in d.i. the kidneys can secrete a urine concentrated in NaCl will also be reported.

Methods used in this type of study have already been described (15). Each rat's fluid exchange was measured daily. The posterior lobe of the hypophysis was removed by the ventral approach with minimal damage to the anterior lobe (posthypophysectomy). Aggravations of the d.i. state following this operation were accomplished by giving the animal saline drinking solutions.

EXPERIMENTAL

Nephrectomies in diabetes insipidus. After nephrectomy, it is evident that the animal is by no means normal, but it is argued that for a short period thereafter the animal may be considered relatively normal. We have found the rat to survive total nephrectomy, provided there are no other complicating factors, for from 3 to 5 days; it has therefore been assumed that the 'relatively normal period' lasts for one day after the operation. During this period the rats were lively and cheerful; some appetite continued since they ate about one-third the daily ration. The nephrectomies were done usually in a two-stage operation in order to minimize trauma.

TABLE 1. EFFECT OF NEPHRECTOMY ON DIABETES INSIPIDUS

Group No.	No. of rats	Kinds of Operation	Drinking solution	cc. per rat per day during week before nephrectomy	cc. drunk per rat during 24 hours after nephrectomy	Ave. wt. at nephrectomy gm.	Ave. wt. 24 hours after nephrectomy gm.	% change in weight
1	6	Nephrectomized	water	11	16	165	168	+ 2
2	12	Posthypophysectomized	water	11	60 (during day after post-hypophysectomy)	—	—	—
3	6	Nephrectomized and posthypophysectomized at same time	water	10	4	154	146	− 5
4	9	Nephrectomized 2 weeks after posthypophysectomy	water	45	13	162	164	+
5	6	Nephrectomized	0.9% NaCl	15	10	158	169	+
6	7	Posthypophysectomized	0.9% NaCl	13	91 (during day after post-hypophysectomy)	—	—	—
7	6	Nephrectomized and posthypophysectomized at same time	0.9% NaCl	12	12	150	150	0
8	7	Nephrectomized 4 to 10 weeks after posthypophysectomy	0.9% NaCl	43	57	265	313	+18

The effects of the operation on d.i. are shown in table 1. Controls of both types of operation taken singly are shown. Groups 1 through 4 were given water to drink and groups 5 through 8 were given saline. The nephrectomies were done in both the transient phase and the permanent phase of d.i. The rate of fluid ingestion of the week before the nephrectomy indicates

the rate that might be expected postoperatively, if the thirst of the animal were not influenced by the operation. Weight changes are also included.

The table shows that in all groups, except number 8, nephrectomy is followed by a rate of fluid ingestion, with minor variations, that is about normal for the intact rat, viz. about 12 cc. a day. This occurred even though the rat was in the transient phase (group 3) or the permanent phase (group 4) of d.i. and is in entire agreement with Richter's reports (1, 3) of much the same experiment. Taken alone, these experiments indicate that the polyuria of d.i. is primary. But exactly the reverse result was obtained by nephrectomies on rats with a d.i. of the permanent phase aggravated by giving saline to drink (group 8). They continued to drink at the excessive rate previously established, which resulted in an 18% gain in weight in 24 hours.

In one extreme case in group 8, the rat was ingesting about 30 cc. of saline per day. At the nephrectomy it weighed 165 gm. During the first 24 hours, 40 cc. of saline was drunk, during the second 38 cc., and during the third 36 cc., with death occurring at the 82nd hour. This ingestion of a total of 114 cc. of fluid that the animal could not excrete was accompanied by an increase in weight of 111 gm.—up 67%. On autopsy, but little ascitic fluid was found, but a large portion of the ingested saline had evidently been stored subcutaneously. The body wall at the sternum swelled to 16 mm. in thickness (normal is about 4) and a shiny rubbery material was found between muscle and epidermis. On cutting, fluid did not ooze freely from this tissue. Histologic examination showed a massive edema, involving the fat and loose areolar tissues and to a lesser degree the dense connective tissues. One gram of this tissue, when dried to constant weight, shrank to 0.12 gm.

Restriction in fluid intake in diabetes insipidus. Restriction of the intake of water was carried out on a group of 8 rats from the posthypophysectomy until 8 days later. Each rat was given 10 cc. of water twice daily. One rat died on the third day postoperatively, but the other 7 tolerated the treatment quite well. All lost weight, the average being 9.6%; but posthypophysectomized rats given water ad libitum also lose weight over this same period, the average loss of a group of 12 such rats being 9.1%. Evidently, then, even recently operated rats tolerate restriction of the fluid intake quite well, in agreement with other results.

It was noted that even with the restriction, the three phases of d.i. occurred as shown in the records of the animals' urinary output. Thus, in the transient phase, an average of 23 cc. per rat per day was excreted—3 cc. in excess of the intake. In the interphase 2 cc. was excreted and in the permanent phase 16 cc. was excreted. The three phases of d.i. in the rat, therefore, developed independently of the amount of fluid ingested, thus confirming observations in the cat (2) and in the dog (13, 14).

During the day after free access to water was allowed to the rats, they gained an average of 2% in weight. Such a small increase indicates that there was little or no water deficiency or dehydration in the fluid-restricted rats.

Rats in which the d.i. following posthypophysectomy was aggravated by saline drinking solutions were also subjected to fluid restriction. All were drinking 0.9% NaCl. For a month, each was given 7 cc. of this solution to

drink twice a day; this quantity of 14 cc. per day is about what the normal rat consumes. After a month, each rat was given an ad libitum supply of saline to drink. The effects of this treatment on the rats' weights and on their rate of fluid ingestion after the period of restriction are shown in table 2. The average intake per rat per day before the restriction was 60 cc., the individuals varying from 20 to 150 cc.

TABLE 2. EFFECT OF 30 DAYS OF FLUID RESTRICTION ON D.I. RATS

Rat No.	Weights			Average daily intake for week	
	At start of restriction	At end of restriction	24 hours after end of restriction	Preceding restriction	Following restriction
1	205	217	213	20	19
2	202	175	187	28	111
3	212	206	208	30	30
4	312	318	320	33	36
5	252	252	252	33	44
6	225	226	228	39	31
7	234	229	226	55	58
8	230	210	215	60	64
9	226	212	216	68	54
10	210	200	208	90	77
11	266	253	254	104	77
12	238	225	240	150	170
Averages	235	227	235	60	73

From the point of view of weight change, the rats tolerated the restriction fairly well. They lost an average of 8 gm., whereas the healthy rat continues slowly to gain weight. This average, however, is heavily influenced by the 27 gm. weight loss of rat no. 2.

Rat no. 12 had an extreme d.i. of 150 cc. per day, but restricting this to one-tenth the quantity resulted in the relatively slight loss of weight of 13 gm. All of the animals were extremely thirsty, as evidenced by their scrambling to get at the fluid when it was given to them. They usually drank the 7 cc. allotted within 2 minutes. The restriction caused the urinary specific gravity to rise from about 1.008 to 1.030, indicating that they were forced to excrete a concentrated urine.

The period of restriction did not affect the rate of fluid exchange which had been established before the period of restriction, since when given saline ad libitum, the group's average rate of ingestion of fluid continued at the pre-restriction level. One rat, however, no. 2, showed a marked increase.

During the first 24 hours after saline was given ad libitum, the weight increased an average of 3.5%. This small increase hints that a slight degree of dehydration may have been present. During the first 2 hours of free access to saline, an average of 25 cc. per rat was drunk while an average of 10 cc. was excreted. The polydipsia in this case clearly preceded in time the polyuria.

In another study (15) it was shown that if the d.i. rat was given 1.8% NaCl to drink, death was caused in most cases. It was also determined in this study if the d.i. rat could tolerate a restricted 1.8% NaCl intake. Four such

rats were given 10 cc. of 1.8% NaCl twice daily. They were unable to tolerate it, death occurring in about 10 days. The normal rat, however, can tolerate such a restriction or large daily quantities, 35 cc., of such a drinking solution without apparent ill effects.

Measurements of the ability to secrete a concentrated urine. Meyer (16, 17) thought that in d.i. there exists a relative inability of the kidneys to secrete a urine concentrated in NaCl. Such a theory would explain the slow aggravation that follows saline administration (15) and the lethal effect of giving d.i. rats 1.8% NaCl as drinking water (15 and above). It also would account for the fact that the major portion of the large fluid exchange occurring in the permanent phase of d.i. is dependent on ingested NaCl (18). But most workers are agreed that in d.i. the kidneys can secrete a urine concentrated in NaCl and other substances as well. The d.i. animal can be forced to excrete a concentrated urine by restricting its fluid intake (2, 8, 9, 10 and above).

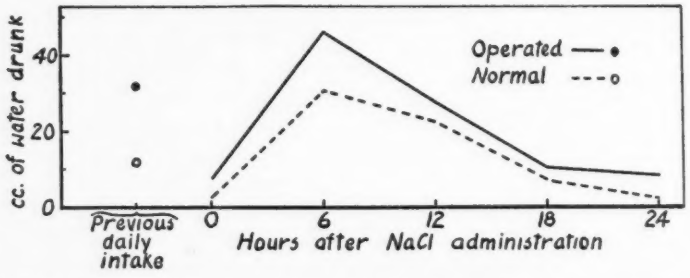


Fig. 1. Diuresis from NaCl administration in normal and posthypophysectomized rats.

Also, experiments on man (19, 20) and experiments on dogs (8, 9) show that an excess of NaCl is excreted in d.i. with about the same efficiency as in the normal animal. This occurs even when the intake is restricted (9). These observations have been confirmed as follows: a group of 9 rats, whose d.i. had been aggravated previously by giving them saline drinking solutions, and a group of 10 normal rats were given 5 gm. per kg. of NaCl by mouth in 30% solution. This quantity of salt borders on the lethal. The quantity of water ingested was then measured every 6 hours, the average intake per rat being shown in figure 2. The rate of water intake at zero was computed by taking a quarter of the average daily intake for the week previous to the salt administration. Only the average rat's intake is shown in figure 2; the urinary output gave the same sort of curve.

Figure 2 shows that from the point of view of the time necessary for the fluid intake to return to normal, the two groups reacted similarly: at 18 hours after the NaCl administration, both had returned almost to the normal rate of fluid ingestion. Both groups, then, had a diuresis of the same duration.

From the point of view of the amount of water ingested during the saline diuresis, the operated animals drank somewhat more than the controls, i.e., 18 hours after the salt administration they had consumed an average of 85 cc. of water whereas the normal animals had consumed a total of 62 cc. But even

so, the operated animals secreted a urine almost as concentrated as the controls: at the peak of the urine excretion (at 6 hours), the specific gravities of operated rats' urine were 1.019 to the normals' 1.022. This was an increase in concentrating power for the operated animals, since in d.i. the specific gravity is about 1.005, usually inversely proportional to the degree of d.i.

By and large we are inclined to minimize these differences and to consider that the d.i. rat can handle an extra load of NaCl with almost the same efficiency as the normal rat. If anything were seriously wrong with the d.i. kidney's ability to handle NaCl, it would give results far different from these.

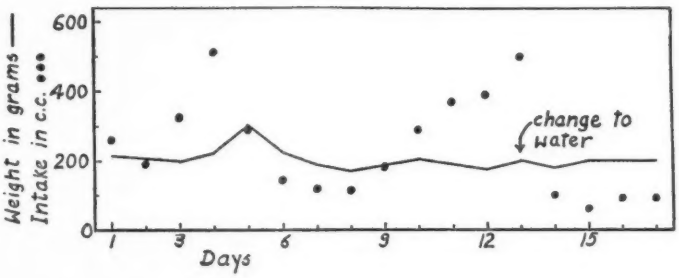


Fig. 2. Rate of ingestion of saline in diabetes insipidus exceeding rate of secretion.

Fluid ingestion exceeding the excretory ability. In one instance, a d.i. rat actually drank 0.9% NaCl faster than its kidney could excrete it. In figure 2, the rat's record is shown. The animal had been posthypophysectomized 7 months previously and unilaterally nephrectomized 2 months previously. In figure 2, it will be seen that it ingested on day 4 a total of 513 cc. of saline when at a weight of 214 gm.; this is equivalent to 2397 cc. per kilo. The weight meanwhile had started to rise, so that by day 5 the rat had gained 100 gm., a 50% increase in weight in 2 days, and evidently the single kidney was falling behind. The drinking then slackened off and the extra weight was rapidly lost. The same excessive drinking then recurred on days 11, 12 and 13, this time with only a slight weight increase. When the rat on day 13 was given water instead of saline to drink, the rate of ingestion dropped at once to low levels. The maximal rate of excretion of fluid of which this animal's kidney was capable appears to be from 2000 to 2500 cc. per kg. per day.

DISCUSSION

There has been much argument about whether in d.i. the polyuria or the polydipsia is primary, but in this report it has been shown that in certain aspects of the condition the polyuria is pretty clearly primary whereas in others the polydipsia is pretty clearly primary. The clearest evidence comes from the experiments involving nephrectomy: in three aspects of d.i. this abolished the polydipsia but in one aspect it did not. Also, the case of one rat is reported in which the excretion rate could not keep up with the ingestion rate. In another paper (15), it was suggested that operative d.i. and sa-

line-aggravated operative d.i. were in some unknown way different. Some of the data presented in this paper support this idea. It looks, from the nephrectomy experiments particularly, as if in operative d.i., uncomplicated by giving saline as drinking solution, the polyuria were primary. On the other hand, if the d.i. is aggravated by giving saline as drinking solution, then the polydipsia appears primary. In the latter case, it might be expected that fluid restriction would be well tolerated and this was found to be true. In the former case, it might be expected that fluid restriction would not be so well tolerated. Although our data do not support this clearly, those of others do (2, 14). As pointed out previously, however, this type of experiment is not decisive.

The only difference between the d.i. in which the polyuria appears primary and that in which the polydipsia appears primary is that in the former water is drunk while in the latter, saline is drunk. Now, in another paper (18), it has been shown that the ingestion of NaCl is responsible for most of the large fluid exchange of both onset and continuance of the permanent phase of d.i. It was therefore suggested that the aberrant water metabolism of d.i. is secondary to some defect in NaCl metabolism; it was also suggested that the reason why excess NaCl aggravates d.i. is that it gives an animal already defective in salt metabolism still more salt to metabolize. Thus, both types of d.i. were suggested to differ only in degree. It is for this reason that the notion that the two types of d.i. differ in kind does not appear very satisfactory. The matter will be elucidated, it is hoped, by studies of the metabolism of Na and Cl in d.i.; these are now in progress.

Kunstmann reported (21) that excessive water drinking led to great and even torturesome thirst. There is a possibility that some such factor may be involved in d.i. Although formal proof that it is not involved has not yet been made, various studies besides this one (2, 10) agree that it does not appear applicable to d.i. If it were working, one might expect that a long period of fluid restriction in d.i. would prevent the development of such a pathologic thirst as Kunstmann reports. But this does not occur in the cat (2) or the dog (10). The month-long restriction of the saline intake of these d.i. rats is equivalent to about 3 years of a human life, but this did not change the thirst of the animals.

Meyer's theory (16, 17) that the d.i. kidney cannot secrete a urine concentrated in NaCl we have found false, in its main aspects, in confirmation of many other studies. Nevertheless, it is felt that the theory is not completely without merit, since giving d.i. rats 1.8% saline to drink, even when restriction also is practiced, results in the animals' deaths (15 and above). If the kidneys were normal in the ability to handle this drinking solution, the animals would surely not have been killed. Also, it was found that the d.i. rat uses somewhat more water than the normal in disposing of a load of salt. Perhaps a degree of hyposthenuria is present in d.i.

Richter (22) has made an interesting comparison of the maximal amounts of diuresis in d.i. in various species. From his records, he reported a d.i. in one rat of 1195 cc. per kg. per day. From this and from experiments attempting to measure the maximum amount of water the normal rat can

handle, he suggested that the maximum quantity of fluid that a rat could excrete per day is about 1100 cc. per kilo. It is considered that this figure is somewhat low since it has often been exceeded in our observation. The single rat which ingested saline faster than it could excrete it is unfortunately not a definitive case since only one kidney was present. On autopsy, this kidney was found to have almost doubled in size. Its excretory rate was exceeded by the ingestion rate only when the intake exceeded about 2000 to 2500 cc. of saline per kg. per day. This rate of ingestion of fluid far exceeds any hitherto reported in the literature when computed on a weight basis. It exceeds only slightly, however, the maximum d.i. reported for man (22, 23) when computed on a basis of equivalent surface areas.

SUMMARY

In some aspects of diabetes insipidus in the rat, the polyuria appears primary while in others the polydipsia appears primary. Thus, total nephrectomy prevents the polydipsia of both the transient and the permanent phases of diabetes insipidus, providing the animals are drinking water. If they are drinking saline and if they have come to the permanent phase of diabetes insipidus, the nephrectomy does not abolish the polydipsia. The case of one rat is reported which ingested saline at a rate faster than it could excrete it, viz. from 2000 to 2500 cc. per kg. per day.

The observation that animals with diabetes insipidus can tolerate fluid restriction fairly well has been confirmed; even after extreme aggravation of the condition with salt, fluid restriction for long periods is not harmful. The observation that in diabetes insipidus the animal can be forced to secrete a urine concentrated in NaCl has also been confirmed.

REFERENCES

1. Richter, C. P.: *Am. J. Physiol.* 112: 481. 1935.
2. Fisher, C., W. R. Ingram and S. W. Ranson: Diabetes Insipidus and the neuro-hormonal control of water balance, etc. Edwards Brothers, Ann Arbor, Mich., 1938.
3. Richter, C. P., and J. F. Eckert: *Am. J. Physiol.* 113: 578. 1935.
4. Klisiecke, A., M. Pickford, P. Rothschild and E. B. Verney: *Proc. Roy. Soc. S.B.* 112: 496. 1933.
5. Gilman, A., and L. Goodman: *J. Physiol.* 90: 113. 1937.
6. Burgess, W. W., A. M. Harvey, and E. H. Marshall: *J. Pharmacol. & Exper. Therap.* 49: 237. 1933.
7. Gersh, I.: *J. Pharmacol. & Exper. Therap.* 52: 231. 1935.
8. Bailey, P., and F. Bremer: *Arch. Int. Med.* 28: 773. 1921.
9. Curtis, G. M.: *Arch. Int. Med.* 34: 801. 1924.
10. Bellows, R. T., and W. P. Van Wagenen: *J. Nerv. & Ment. Dis.* 88: 417. 1938.
11. Weir, J. F., E. E. Larson and L. G. Rowntree: *Arch. Int. Med.* 29: 306. 1922.
12. Bellows, R. T.: *Am. J. Physiol.* 123: 14. 1938.
13. Camus, J., and G. Roussy: *Compt. rend. Soc. de biol.* 75: 483. 1914.
14. Bourquin, H.: *Am. J. Physiol.* 79: 362. 1926.
15. Swann, H. G., and B. J. Penner: *Endocrinology* 24: 253. 1939.
16. Meyer, E.: *Deutsch. Arch. f. klin. Med.* 83: 1. 1905.
17. Meyer, E.: *Ztschr. f. klin Med.* 74: 352. 1912.
18. Swann, H. G.: In press. *Am. J. Physiol.*, 1939.
19. Finkelnburg, R.: *Deutsch. Arch. f. klin. Med.* 100: 33. 1910.
20. Socin, C.: *Ztschr. f. klin. Med.* 78: 294. 1913.
21. Kunstmann, R. : *Arch. f. exper. Path. u. Pharm.* 170: 701. 1933.
22. Richter, C. P.: *Am. J. Physiol.* 122: 668. 1938.
23. Whitehead, R. W., and W. Darley: *Endocrinology* 15: 286. 1931.

ADRENALECTOMY AND COITUS-INDUCED OVULATION IN THE RABBIT AND CAT[1]

H. B. FRIEDGOOD

From the Department of Physiology in the Harvard Medical School
BOSTON, MASSACHUSETTS

RABBITS USUALLY ovulate within 10 to 12 hours post-coitum; but ovulation can be prevented if the anterior hypophysis is removed within 60 to 90 minutes after mating. This signifies that the anterior hypophysis is stimulated by coitus to secrete its gonadotropic hormones within the stipulated period (1, 2). The precise mechanism by means of which this is accomplished is as yet unknown, although the evidence suggests that in the rabbit a neural stimulus, initiated by coitus, passes to the hypothalamus and through the infundibular stalk to the anterior hypophysis (3, 4, 5, 6). Although Harris and Brooks agree on the general course of this pathway, they are not in accord with reference to the systemic and endocrine effects of stalk-section. Harris' rabbits refused to mate and their gonads atrophied after transection of the stalk, whereas Brooks reported that in his rabbits the gonads were histologically normal and that the mating behavior was unaltered after a similar operation. In any event, this interpretation of the available data cannot be accepted without reservation until there is undisputed histological proof of these nervous connections. Such evidence is still lacking. Hair (7) found many nerve fibers of other than sympathetic origin in the anterior lobe of the cat. Brooks (6), who reported similar observations for the rabbit, stated that nerve fibers "were not extremely numerous and seemed to vary in quantity in different animals but were found in all parts of the lobe." He was unable to trace them into the infundibular stalk. Rasmussen (8), on the other hand, found only meager and, in his opinion, insignificant evidence of nerve fibers in the anterior lobe of several species of mammals.

The problem of hypophyseal innervation is complicated still further by other observations. Greep (9) reported that the normal ovarian cycle, estrous rhythm and reproductive functions were maintained in the rat after complete hypophysectomy if a male pituitary was transplanted successfully into the pituitary fossa. His findings argue either in favor of a hormonal stimulus to the gonadotropic activity of the anterior hypophysis or an intrinsic functional rhythm of the hypophyseal gonadotropic cells.

The present investigation was begun in 1936 at a time when the mechanism of coitus-induced pituitary stimulation was still in a highly speculative stage. The experiments were devised in order to test the possibility of a hormonal link in the chain of events which are initiated by coitus and terminate in activation of the gonadotropic functions of the anterior pituitary

[1] Aided by a grant from the Committee for Research in Problems of Sex, National Research Council

gland. The adrenal cortex was made the first object of investigation in this connection because of suggestive reports from various sources which indicated that there might be a close physiological relationship between the adrenal cortex and the gonads.

The first experiments were made with rabbits during the months of August, September and October, 1936. The technic used in these operations differed from the usual procedures for adrenalectomy. A midline incision was made from the top of the xyphoid to the level of the lower pole of the left kidney. The latter was then retracted downward gently, and the liver and stomach packed off with warm saline-soaked sponges. The peritoneal covering of the left adrenal was then incised, the adrenal freed from the surrounding tissue and its pedicle, including its blood vascular supply, tied off as far away from the gland as possible. The adrenal was now removed completely intact. The right adrenal was then approached from the left side of the vena cava. After the abdominal aorta was located and retracted gently to the left (a little above the original level of the left adrenal), the right adrenal was exposed to view by rolling the vena cava from left to right with the aid of small hemostats carefully placed at the upper and lower poles of the right adrenal. Ligatures were then applied to the connective tissue held by both hemostats, and with the additional traction gained by this procedure, the right adrenal was brought completely into view and freed as far as possible from the adventitia of the vena cava. A third ligature was then placed between the gland and the vena cava and slowly tightened over the wall of the vein as far away from the gland as one could get. The adrenal was then removed completely intact. Occasionally a tiny bit of adrenal tissue got caught in the ligature. In such circumstances, the stump was cauterized

TABLE 1

Rabbit no.	No. of matings	Type of operation	Anesthesia	Time of autopsy after 1st mating	Ovulation	Cortin[2] in cc. per day
				hr.		
R1	4	None	0	10	+	0
R2	4	None	0	10	+	0
R3	3	None	0	11	+	0
R4	2	None	0	11	+	0
R5	4	None	0	11¼	+	0
R6	5	None	0	11½	+	0
R7	4	None	0	11¾	+	0
R8	2	None	0	12	+	0
R9	3	None	0	13	+	0
R10	4	None	0	15	+	0
R11	4	None	0	15	+	0
R12	3	None	0	15	+	0
R13	2	None	0	15	+	0
R14	3	None	Urethane	11½	+	0
R15	3	None	Urethane	11¾	+	0
R16	1	Left nephrectomy, experimental mesenteric thrombosis	Urethane	17	+	0
R17	2	Left nephrectomy	Ether	48	+	0
R18	0	None	0	24	0	15
R19	0	None	0	24	0	15

[2] I am indebted to Dr. David Klein of the Wilson Laboratories for the cortin used in these experiments.

carefully. This operative approach is possible because the right adrenal lies in contact with the posterior wall of the vena cava and is not found to the right of it as one might anticipate.

Although the rabbits seem to stand this operation well, almost half of them succumb to the typical effects of adrenalectomy within the first 24 hours. This coincides with the experience of Firor and Grollman (10), who found that 47.7% of their rabbits died in cortico-adrenal insufficiency in less than 24 hours after operation. In contrast to these results, all of the 5 rabbits adrenalectomized by Brooks and Page (11) survived for 2 to 6 days.

TABLE 2

Rabbit no.	No. of matings within 5 to 10 min.	Time of adrenalectomy after 1st mating		Anesthesia	Time of autopsy after 1st mating	Ovulation	Cortin in cc.
		hr.	*min.*		*hr.*		
RA1	3		52	Urethane	10	0	0
RA2	2	4	11	Urethane	12	0	0
RA3	4	1	23	Urethane	12¼	0	0
RA4	1	4	30	Urethane	12⅛	+	0
RA5	5		39	Ether	15	+	0
RA6	4		41	Ether	19	+	14
RA7	3		52	Urethane	19½	0	0
RA8	5		44	Ether	23½	0	0
RA9	4	1		Ether	23¾	+	15
RA10	2		32	Ether	24¼	+	11
RA11	3		30	Ether	24¼	+	10
RA12	1		39	Ether	46	+	0

All of 13 normal estrous rabbits ovulated within 10 to 15 hours post-coitum (table 1). Neither non-specific abdominal operations nor moderately light anesthesia for approximately 1 hour interfered with the expected post-coital ovulatory response in 4 other rabbits (table 1). Cortin in large doses did not induce ovulation in control experiments (table 1), and was not a factor, therefore, in its occurrence under other experimental conditions (table 2). However, of the 11 adrenalectomized animals whose ovaries were examined within 10 to 24 hours after mating only 6 ovulated (table 2). These results were tentatively interpreted to indicate that the adrenals were unessential to the mechanism responsible for coital stimulation of the anterior pituitary gland. The variability of the results (table 2) made it advisable to repeat these experiments under more ideal conditions before drawing any final conclusions. It was thought that either the completeness of adrenalectomy or the occurrence of accessory adrenal tissue might account for the lack of consistent results. Accordingly, the experiments were repeated using the cat as an experimental animal. The cat was chosen for two reasons: *a*), it, like the rabbit, normally ovulates only after coitus; (*b*), it is technically easy to remove both adrenals completely in the cat, whereas the anatomical position of the rabbit's right adrenal makes adrenalectomy a particularly difficult surgical procedure.

Nine cats were adrenalectomized within 17 to 48 minutes after mating and autopsied from 27 to 168 hours later; and 3 cats were adrenalectomized 6 hours or more post-coitum and autopsied between 49¾ and 96 hours later.

Two mated cats were exposed to prolonged anesthesia alone and their ovaries were examined at 48½ and 115 hours later. None of the 9 cats in the first group ovulated; all 3 cats in the second group ovulated; and both of the cats which were simply anesthetized after mating also ovulated (tables 3 and 4). Since the normal cat ovulates within 25 to 36 hours post-coitum (12, Friedgood, unpublished data), it was concluded that the adrenals are essential for post-coital ovulation in the cat (13). This interpretation has been

TABLE 3

Cat. no.	No. of times mated	Time of adrenalectomy after mating		Autopsy. no. of hr. after mating	Ovulation	Cortin in cc. per day
		hr.	*min.*			
C1[1]	2		17	27	0	0
C2	1		25	46½	0	0
C3[1]	2		25	168	0	8
C4[1]	1		25	55	0	0
C5[1]	2		26	44¼	0	0
C6[1]	1		30	114	0	0
C7	3		37	60¼	0	4
C8	1		37	114	0	8
C9	2		48	28½	0	0
C10	1	6		54	+	8
C11	2	6¼		49¾	+	8
C12	3	9		96	+	0

[1] Unilateral adrenalectomy 3 to 6 weeks prior to present operation.

TABLE 4

Animal no.	No. of times mated	Nature of operation	Start of anesthesia after first mating	Duration of anesthesia (ether)	Time of autopsy after 1st mating	Ovulation
			min.	*min.*	*hr.*	
CE1	3	None	25	35	48½	+
CE2	3	None	26	35	115	+
CL1	4	Blank laparatomy	20	23	120	0
CS1	2	Partial splenectomy	25	38	48	0
CS2	2	Partial splenectomy	25	40	46	0
CS3	3	Partial splenectomy	25	33	72	0
CN1	3	Unilateral nephrectomy	17	30	72	0
CN2	3	Unilateral nephrectomy	15	35	48	+
CN3	2	Unilateral nephrectomy	24	15	96	+
CN4	2	Unilateral nephrectomy	25	50	67¼	0
CN5	3	Unilateral nephrectomy	25	40	168	0
CN6	3	Unilateral nephrectomy	29	37	72	0
CC1	4	Left half of celiac ganglion removed. Right adrenalectomy	25	49	48	+
CC2	4	Left half of celiac ganglion removed. Right adrenalectomy	34	35	45	0
CC3	3	Left half of celiac ganglion removed. Right adrenalectomy	25	40	70	0
CC4	4	Left half of celiac ganglion removed. Right adrenalectomy	21	25	72	+
CC5	3	Left half of celiac ganglion removed. Right adrenalectomy	20	20	52	0

corrected in the light of a more extensive series of control observations which became possible a year later when the estrous season for cats returned.

This additional experience (table 4) showed that a variety of abdominal operations on 15 cats interfered in most instances with the occurrence of post-coital ovulation. Such surgical procedures included a blank laparotomy, unilateral nephrectomy, partial splenectomy, and unilateral adrenalectomy in addition to removal of a portion of the celiac ganglion. The results obtained in this new series of controls now warrant the conclusion that some factor other than adrenalectomy was responsible for the lack of ovulation in the first group of cats. It is thus apparent that although the ovaries of the normal cat and rabbit react similarly to the stimulus of coitus they do not do so if the animals are subjected to a laparotomy soon after coitus takes place. The rabbit ovulates in spite of drastic abdominal operations (table 1), whereas the cat usually does not (tables 3 and 4).

Within recent months I have had the opportunity of sectioning and making a painstaking search through serial sections of the sites of adrenalectomy in the rabbits operated on in 1936. It was found that adrenalectomy was complete in each instance and that the data were therefore trustworthy in spite of their variability. To what, then, may the latter be reasonably attributed?

A possible answer to this question was suggested by the observation that only one of 4 adrenalectomized rabbits ovulated within 10 to 12 hours and that the ovulation points in the ovaries of rabbits RA9 and RA10 were unusually fresh (table 2). Since these two rabbits were autopsied at 23¾ and 24¼ hours post-coitum, the occurrence of ovulation was probably significantly delayed by the adrenalectomy, for it is well recognized that the normal estrous rabbit ovulates within 10 to 12 hours after mating. Heape (14) reported that maturation of the ovum with polar body formation takes place at about 9 hours and that ovulation occurs at approximately 10 hours post-coitum. Hammond and Marshall (15) and Marshall and Verney (16) also placed the time of ovulation at 10 hours after mating. Fee and Parkes (1) reported that their rabbits ovulated within 10 to 12 hours post-coitum. My own experience with normal rabbits (table 1) has been similar to those of the above-mentioned investigators.

Adrenalectomy is also known to delay the time of ovulation in cats, when ovarian stimulation is initiated by the administration of FSH and LH (17). Although this may have some bearing on the problem which is being discussed, it is mentioned with reservation because of the difficulty of transferring the results of observations from one species to another.

These data suggest that some of the adrenalectomized rabbits which were autopsied within 10 to 23¼ hours post-coitum were killed before they had a chance to ovulate. This may account for those adrenalectomized animals which did not ovulate (table 2), and also satisfactorily explains the uniformly positive results obtained by Brooks and Page (11), who reported that all their rabbits were found to have ovulated when examined 2 to 6 days post-coitum. In this connection one must also bear in mind the possibility that an occasional estrous rabbit fails to ovulate even after normal coitus (18, Freidgood, unpublished data).

SUMMARY

Thirteen estrous rabbits were mated and subsequently autopsied at intervals between 10 and 15 hours post-coitum. All of them ovulated. In a second series of experiments, 2 estrous rabbits were lightly anesthetized with urethane within 9 minutes after mating, and 2 others were subjected to a left nephrectomy and an experimentally induced mesenteric thrombosis. These rabbits also ovulated. In a third group there were 2 estrous rabbits which were given 15 cc. of cortin in 24 hours in 3 equally-spaced doses of 5 cc. each; neither ovulated (table 1).

Twelve estrous rabbits were adrenalectomized bilaterally within ½ to 4½ hours after mating. Seven of them ovulated. Of the 5 which did not ovulate, 3 were autopsied within 10 to 12¼ hours post-coitum and this may have been too early to detect a somewhat delayed ovulation (table 2).

It is concluded that the adrenals are not essential to the mechanism responsible for post-coital ovulation in the rabbit.

The normal cat ovulates within 25 to 36 hours post-coitum. Nine estrous cats were adrenalectomized completely within 17 to 48 minutes post-coitum and autopsied 27 to 168 hours later. None of them ovulated. Three cats in a second group were adrenalectomized within 6 to 9 hours after mating and examined 54 to 96 hours later. All of them ovulated. In a third group there were 2 estrous cats which were anesthetized with ether for 35 minutes after having been mated; both ovulated. Fifteen estrous cats in a fourth group were subjected to various abdominal operations after mating (blank laparotomy, partial splenectomy, unilateral nephrectomy and right adrenalectomy with partial ablation of the celiac ganglion). Only 4 of these 15 cats ovulated (tables 3 and 4).

Although the ovaries of both the estrous cat and rabbit respond with ovulation to the stimulus of coitus, they do not react similarly if the animals are subjected to a laparotomy soon after mating. The rabbit ovulates in spite of major abdominal operations, whereas the cat usually does not. Because of the large percentage of cats which did not ovulate after non-specific abdominal operations, it is probable that adrenalectomy per se does not influence the progress of post-coital ovulation in the estrous cat.

REFERENCES

1. Fee A. R., and A. S. Parkes: *J. Physiol.* 67: 383. 1929.
2. Smith, P. E., and W. E. White: *J. A. M. A.* 97: 1861. 1931.
3. Westman, A., and D. Jacobsohn: *Acta Obst. et Gynec. Scandinav.* 17: 235. 1937.
4. Haterius, H. O., and A. J. Derbyshire: *Am. J. Physiol.* 119: 329. 1937.
5. Harris, G. W.: *Proc. Roy. Soc., London, s.B,* 122: 374. 1937.
6. Brooks, C. McC.: *Am. J. Physiol.* 121: 157. 1938.
7. Hair, G. W.: *Anat. Rec.* 71: 141. 1938.
8. Rasmussen, A. T.: *Endocrinology* 23: 263. 1938.
9. Greep, R. O.: *Proc. Soc. Exper. Biol. & Med.* 34: 754. 1936.
10. Firor, W. M., and A. Grollman: *Am. J. Physiol.* 103: 686. 1933.
11. Brooks, C. McC., and S. W. Page: *Endocrinology* 22: 613. 1938.
12. Greulich, W. W.: *Anat. Rec.* 58: 217. 1934.
13. Friedgood, H. B.: *Science* 86: 84. 1938.
14. Heape, W.: *Proc. Roy. Soc., London, s.B,* 76: 260. 1905.
15. Hammond, J., and F. H. A. Marshall: Reproduction in the rabbit. Edinburgh, 1925.
16. Marshall, F. H. A., and E. B. Verney: *J. Physiol.* 85: 12P. 1935.
17. Friedgood, H. B., and M. A. Foster: *Am. J. Physiol.* 123: 237. 1938.
18. Brooks, C. McC. *Am. J. Physiol.* 120: 544. 1937.

PHYSIOLOGICAL CHANGES IN THE ANTERIOR HYPOPHYSIS OF VITAMIN A-DEFICIENT RATS

T. S. SUTTON AND B. J. BRIEF

From the Department of Dairy Industry, Ohio Agricultural Experiment Station, and the Department of Anatomy, The Ohio State University

COLUMBUS, OHIO

SHORTLY AFTER the hypophysis was shown to be the source of gonad stimulating hormone(s), attempts were made to demonstrate that the production of these hormones could be modified. The gonad stimulating potency of the gland can be demonstrated and assayed by implanting or injecting it into immature test animals thus superimposing the effect of the implanted or injected gland upon the impotent gland of the test animal, effective dosages being marked by definite changes in the organs of reproduction.

Using ovarian weights as a measurement of the effects of the treatment Engle (1) and Evans and Simpson (2) demonstrated that the hypophyses from castrate animals were much more potent than those from normal animals. Evans and Simpson also showed that the hypophyses from cryptorchid animals evoked a reaction intermediate between that caused by glands from normals and castrates. Similarly, Mason and Wolf (3) have produced evidence that a dietary deficiency of vitamin A caused an increase potency in pituitaries of both castrate and non-castrate rats.

Smith, Severinghaus, and Leonard (4) demonstrated that the increased potency of the castrate rabbit hypophyses was associated with an increase in the basophile cells of the hypophysis.

In a previous paper (5), data were presented showing a marked increase in the basophilic cells of the hypophysis in vitamin A deficiency. To demonstrate whether or not a relationship exists between the increased number of basophile cells in the hypophysis of the vitamin A-deficient animal and the production of gonadotropic hormones, we decided to assay the pituitaries for gonadotropic potency.

The early workers in pituitary assays, as already pointed out, used ovarian weights as a measure of the stimulation produced by the injection or implantation. The stimulated ovary, however, not only increases in weight but also increases in the production of its hormones. Since there may be a considerable increase in hormone production by the ovarian cells without a marked increase in ovarian cell proliferation, it does not necessarily follow there is a close relationship between ovarian hormone output and ovarian weight especially in the lower ranges of stimulation. The ovarian hormones show their presence by their effects on the uterus and vagina. Levin and Tyndale (6) injected immature mice with gonadotropic substance and measured the ovarian stimulation indirectly by measuring the increase in uterine

weights. They were able to show that this method of measurement is three to five times as sensitive for the assay of stimulation as is the ovarian weight method. Heller, Lauson, and Severinghaus (7) used the weight of the immature rat uterus as an assay measurement and confirmed the work of Levin and Tyndale. They make a further statement that this method is simpler and "practically as sensitive as a method based on vaginal histology." Contrary to this last statement Marrian and Parkes (8) and Szarka and Kurtz (9) state that the amount of hormone required to produce uterine changes in test animals is many times that required to produce vaginal changes.

We therefore decided to use the vaginal changes in the immature white mouse for measuring stimulation in our assay.

MATERIALS AND METHODS

The procedure used in the assay is as follows. After the animal from which the hypophysis was to be analyzed had been deeply anaesthetized it was exsanguinated in order to keep the operative field clear of blood. The top of the skull was removed, the brain scooped out and the dura mater reflected so that the pituitary gland laid free in the skull. The pituitary was then transferred to a weighed porcelain crucible and the posterior lobe removed by blunt dissection. It was found that the posterior lobe could be easily freed from the rest of the hypophysis by merely drawing it away with a needle. The vessel and its contents were then immediately weighed. The anterior lobe was then macerated with a glass rod, 1 cc. of distilled water added to the macerated tissue and the whole thoroughly mixed. Distilled water was used as the vehicle in order to rupture, under the influence of osmotic pressure, any cells which might have escaped mechanical disruption. The rupture of the cells frees the contained hormones and precludes the possibility of the cell 'taking' and actively resuming secretion in the test animal. The suspension was then taken up in a tuberculin syringe and a measured quantity injected subcutaneously into the test animal. The test animals were female white mice, 18 days old at the time of injection. When possible to do so, pituitaries from normal animals and vitamin A-deficient animals were injected into individuals in the same litter of mice, to minimize the effect of possible litter variations. One animal was saved from each litter as an uninjected control. Ninety-six hours after injection the mice were killed and ovaries, uterus, and vagina removed for sectioning.

The vaginas from a number of mice were sectioned longitudinally. It was found that the typical reaction was constant over the entire epithelium except near the internal os and near the external opening. We therefore routinely used the middle third of the vagina as our test region and sectioned it transversely for the sake of ease of manipulation. The histological changes of the vagina were divided into 4 stages, modified somewhat from those used by Fluhmann (10) in his estrin assays. Reaction 1 was that of the uninjected control. The vaginal epithelium was 2 cell layers thick and the superficial layer was either cuboidal or columnar. Reaction 2 showed stratification with the superficial layer mucified and usually columnar. In reaction 3 the superficial stratum was squamous and in reaction 4 it was keratinized. Reaction 2 is the earliest reaction attributable to the ovarian hormones since they are of the nature of growth hormones stimulating cellular proliferation. Typical reactions are illustrated in figures 1, 2, 3 and 4.

Since pituitary weights were recorded and measured portions of the one cubic centimeter suspensions injected, it was possible to calculate the weight of pituitary substance injected.

The rats used as pituitary donors were placed on a diet meeting the U.S.P. specifications for a vitamin A free diet except that unextracted Argentine casein was used. This diet contains sufficient vitamin A to provide for growth over a period several

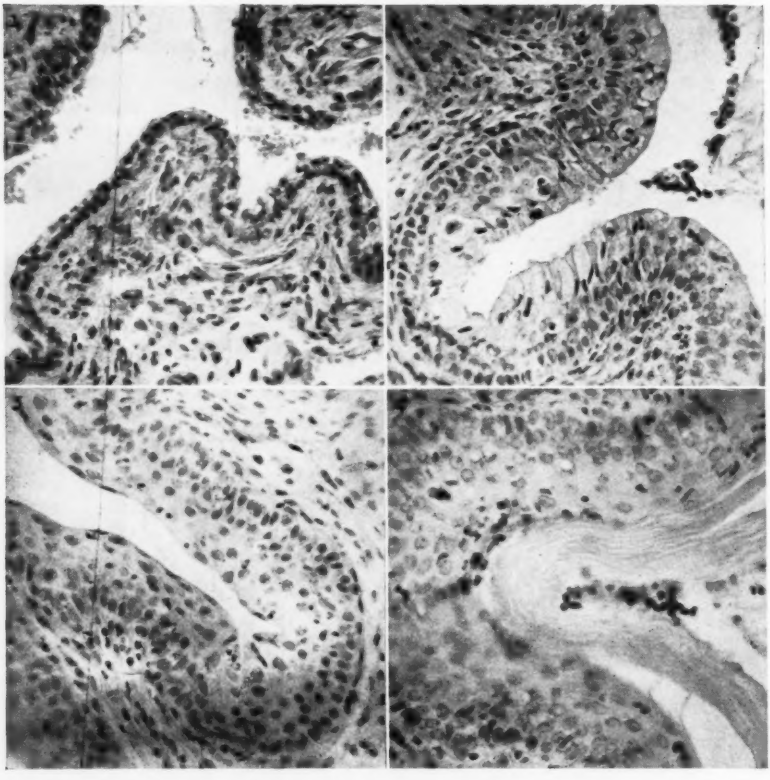


Fig. 1. THE NORMAL VAGINAL EPITHELIUM OF A 22-DAY-OLD MOUSE. This is quite typical for the control animals used in this experiment and is designated reaction no. 1. Fig. 2. THIS REPRESENTS A TYPICAL NO. 2 REACTION. The epithelium consists of more than two layers of cells and the border layer is columnar and mucified. Fig. 3. A NO. 3 REACTION, THE EPITHELIUM IS STRATIFIED WITH A SQUAMOUS SURFACE LAYER. Fig. 4. THE REACTION NO. 4 IS TYPIFIED BY A STRATIFIED SQUAMOUS EPITHELIUM WITH A KERATINIZED SURFACE LAYER.

weeks longer than when alcohol extracted casein is used. Pituitaries from these animals were assayed after mild to moderate symptoms of vitamin A deficiency developed. The normal animals were from our stock colony and their pituitaries were assayed when they were of ages comparable to those of the vitamin A-deficient animals.

RESULTS

The results of these assays are presented in tables 1, 2, 3, and 4 and summarized in table 5. Pituitary injections were made at levels higher than those presented in these tables. As a matter of fact groups of 10 assay animals each were treated with graded dosages of pituitaries beginning at levels of

0.5 of the total anterior pituitary of the donor animals and progressed downward at levels of 0.4, 0.3, etc. At the levels in which more than 0.3 of the total pituitaries of the donor animals were injected the results were some-

TABLE 1. ASSAY RESULTS OBTAINED WHEN 0.3 OF THE ANTERIOR LOBE OF RAT PITUITARIES WAS INJECTED

Pituitaries from normal ♂'s				Pituitaries from normal ♀'s				Pituitaries from Vitamin A deficient ♀'s			
Assay mouse	Total wt. of rat pituitary	Wt. of pituitary injected	Vaginal reaction	Assay mouse	Total wt. of rat pituitary	Wt. of pituitary injected	Vaginal reaction	Assay mouse	Total wt. of rat pituitary	Wt. of pituitary injected	Vaginal reaction
no.	mg.	mg.		no.	mg.	mg.		no.	mg.	mg.	
59	—	—	3	257	5.1	1.53	2	106	3.9	1.17	3
147	3.5	1.05	3	259	4.4	1.32	3	113	5.8	1.74	3
149	3.2	0.96	3	260	3.6	1.08	2	137	2.8	0.84	3
152	3.2	0.96	3	261	6.2	1.86	3	138	3.3	0.99	3
154	3.8	1.14	3	262	4.4	1.32	3	139	3.6	1.08	3
155	3.9	1.17	3	265	5.1	1.53	2	141	3.2	0.96	3
157	3.2	0.96	3	267	3.2	0.96	2	145	3.5	1.05	3
158	2.4	0.72	2	269	5.0	1.50	2	148	3.2	0.96	3
159	3.0	0.90	3	270	4.8	1.44	2	150	3.0	0.90	3
175	2.4	0.72	2	274	3.8	1.14	1	153	3.8	1.14	3
Ave.	3.17	0.95	2.7	Ave.	4.56	1.368	2.2	Ave.	3.61	1.083	3

TABLE 2. ASSAY RESULTS OBTAINED WHEN 0.2 OF THE ANTERIOR LOBE OF RAT PITUITARIES WAS INJECTED

Pituitaries from norma ♂'s				Pituitaries from vitamin A deficient ♂'s				Pituitaries from normal ♀'s				Pituitaries from vitamin A deficient ♀'s			
Assay mouse	Total wt. of rat pituitary	Wt. of pituitary injected	Vaginal reaction	Assay mouse	Total wt. of rat pituitary	Wt. of pituitary injected	Vaginal reaction	Assay mouse	Total wt. of rat pituitary	Wt. of pituitary injected	Vaginal reaction	Assay mouse	Total wt. of rat pituitary	Wt. of pituitary injected	Vaginal reaction
no.	mg.	mg.		no.	mg.	mg.		no.	mg.	mg.		no.	mg.	mg.	
243	3.6	0.72	2	208	3.6	0.72	3	263	4.9	0.98	1	178	2.5	0.50	3
244	3.7	0.74	3	209	2.5	0.50	3	268	4.1	0.82	1	179	1.9	0.38	3
246	3.3	0.66	3	211	1.9	0.38	3	271	5.5	1.10	1	181	2.1	0.42	2
247	3.4	0.68	3	212	2.1	0.42	3	273	6.3	1.26	1	182	3.2	0.64	2
248	4.1	0.82	3	214	2.5	0.50	3	275	3.4	0.68	1	184	3.7	0.74	3
250	3.4	0.68	3	216	2.7	0.54	3	309	5.7	1.14	1	203	3.2	0.64	3
252	2.7	0.54	3	218	2.6	0.52	3	310	6.0	1.20	1	205	5.0	1.00	3
253	3.9	0.78	3	219	2.4	0.48	3	312	4.2	0.84	1	206	3.1	0.62	3
255	3.3	0.66	3	221	3.1	0.62	4	313	5.7	1.14	2	280	4.4	0.88	2
256	4.2	0.84	1	223	2.1	0.42	3					281	5.0	1.00	3
				224	2.3	0.46	2								
				225	1.9	0.38	3								
Ave.	3.56	0.712	2.7	Ave.	2.47	0.495	3.0	Ave.	5.09	1.01	1.1	Ave.	3.41	0.68	2.7

TABLE 3. ASSAY RESULTS OBTAINED WHEN 0.1 OF THE ANTERIOR LOBE OF RAT PITUITARIES WAS INJECTED

Pituitaries from normal ♂'s				Pituitaries from vitamin A deficient ♂'s				Pituitaries from vitamin A deficient ♀'s			
Assay mouse	Total wt. of rat pituitary	Wt. of pituitary injected	Vaginal reaction	Assay mouse	Total wt. of rat pituitary	Wt. of pituitary injected	Vaginal reaction	Assay mouse	Total wt. of rat pituitary	Wt. of pituitary injected	Vaginal reaction
no.	mg.	mg.		no.	mg.	mg.		no.	mg.	mg.	
276	4.9	0.49	2	227	2.0	0.20	2	282	2.7	0.27	2
303	5.4	0.54	3	228	3.6	0.36	3	286	5.4	0.54	2
304	5.3	0.53	3	278	3.4	0.34	3	287	3.9	0.39	2
305	4.7	0.47	3	284	1.7	0.17	3	290	2.6	0.26	2
307	4.9	0.49	2	285	3.1	0.31	3	294	2.2	0.22	2
308	5.1	0.51	2	289	3.6	0.36	2	297	2.4	0.24	2
314	4.4	0.44	2	292	2.6	0.26	3	298	4.5	0.45	2
316	4.4	0.44	2	293	2.8	0.28	2	301	2.9	0.29	1
317	5.4	0.54	2	296	2.3	0.23	3	320	2.6	0.26	2
319	4.3	0.43	2	299	2.9	0.29	3	322	2.3	0.23	2
								323	1.9	0.19	1
Ave.	4.88	0.49	2.3	Ave.	2.8	0.28	2.7	Ave.	3.03	0.30	1.8

what inconsistent. These inconsistencies arose from the fact that when these larger dosages were given the vaginal epithelium itself would frequently present the appearance of a No. 3 reaction while the contents of the lumen

would indicate that the reaction had passed through the cornified stage, (No. 4 reaction). However, in recording the results, the reaction was never given a No. 4 rating unless the cornified layer was attached or laying in close

TABLE 4. ASSAY RESULTS WHEN 0.05 OF THE ANTERIOR LOBE OF VITAMIN A DEFICIENT MALE RAT PITUITARIES WAS INJECTED

Assay Mouse	Total wt. of rat pituitary	Wt. of pituitary injected	Vaginal reaction
no.	mg.	mg.	
325	2.4	.12	2
326	2.4	.12	1
327	3.0	.15	2
328	3.0	.15	2
329	2.7	.13	2
331	3.0	.15	2
332	2.7	.13	1
334	3.0	.15	2
Ave.	2.77	.138	1.75

TABLE 5. SUMMARY OF PITUITARY ASSAYS

Donor animals	Amount of anterior pituitary injected		Average vaginal reaction of mouse recipient
	Portion of total gland injected %	Av. wt. of gland injected mg.	
10 Normal ♂'s	30	1.28	2.9
10 Normal ♀'s	30	1.37	2.2
10 Vit. A Def. ♀'s	30	1.08	3.0
10 Normal ♂'s	20	0.71	2.7
12 Vit. A Def. ♂'s	20	0.49	3.0
9 Normal ♀'s	20	1.01	1.1
10 Vit. A Def. ♀'s	20	0.68	2.7
10 Normal ♂'s	10	0.49	2.3
10 Vit. A Def. ♂'s	10	0.28	2.7
11 Vit. A Def. ♀'s	10	0.30	1.8
8 Vit. A Def. ♂'s	5	0.138	1.75
	78 uninjected control mice		1.07

proximity to the surface of the epithelium. Handling the material in this way tended to minimize the differences in potency between the groups injected at the higher levels. These differences in potency became apparent in a striking manner when smaller dosages were given. For instance a comparison of the data which were obtained by injecting pituitaries from normal and vitamin A-deficient males at the 0.3 level showed no difference in the average reaction. However, it was quite evident that in several cases in the vitamin A-deficient group the vaginal epithelium of the test mouse had passed through a cornified state as judged by the contents of the lumen. Yet in following our arbitrary decision to consider only the adherent epithelium itself in judging the degree of stimulation these were given at No. 3 rating.

From our experience with this technic of assaying pituitaries for gonadotropic activity we feel that levels of administration which will give average reactions between 2 and 3 are most desirable.

An inspection of the data presented in the accompanying tables reveals some interesting facts. The potency of pituitary substance from normal males is greater than that from normal females. The magnitude of this difference is illustrated by the fact that an average of 0.71 mg. of pituitary substance from normal males gave an average reaction of 2.7 while an average of 1.37 mg. from normal females gave an average reaction of 2.2.

The pituitaries of vitamin A-deficient animals of either sex were more potent for gonadotropic substance than those from normal animals of the same sex. Moreover, pituitary substance from vitamin A-deficient males had the greatest potency. An average reaction of 2.7 was obtained from 0.28 mg. of pituitary substance from vitamin A-deficient males while it took 0.68 mg. from vitamin A-deficient females and 0.71 mg. from normal males to produce the same reaction. The sensitivity of the assay technic is illustrated by the response obtained when 0.14 mg. of pituitary substance from vitamin A-deficient males was injected. This average reaction of 1.75 can hardly be considered insignificant when the average of 78 uninjected control mice was 1.07. That is to say that only 5 out of 78 mice used as controls in the entire experiment had a vaginal epithelium that could be given a No. 2 rating.

These differences in potency are in agreement with what might be expected from the differences in cellular composition. In other words, those glands containing the higher percentages of basophilic (beta) cells gave reactions showing greater gonadotropic potency.

This study provides further evidence that vitamin A deficiency exerts a direct damage on the gonads. The increase in gonadotropic hormone elaboration represents a compensatory change in the hypophysis similar to that which occurs following castration.

SUMMARY

A technic for the assay of gonadotropic hormones is described. The degree of gonad stimulation is measured by the changes in the vaginal epithelium. The sensitivity of this technic is illustrated by the fact that 0.14 mg. of anterior pituitary substance gives a measurable stimulation.

Using this technic, anterior pituitaries from normal and vitamin A-deficient rats were assayed for gonadotropic activity. The data obtained show that pituitary substance from normal males has greater gonadotropic activity than that from normal females. In vitamin A deficiency the gonadotropic hormone content of the pituitary is increased. This increase in potency is greater in the male than in the female.

REFERENCES

1. Engle, E. T.: *Am. J. Physiol.* 88: 101. 1929.
2. Evans, H. M., and M. E. Simpson: *Am. J. Physiol.* 89: 371. 1929.
3. Mason, K. E., and J. M. Wolfe: *Anat. Rec.* 45: 232. 1930.
4. Smith, P. E., A. E. Severinghaus and S. L. Leonard: *Anat. Rec.* 57: 177. 1933.
5. Sutton, T. S., and B. J. Brief: *Endocrinology* 23: 211. 1938.
6. Levin, L., and H. H. Tyndale: *Endocrinology* 21: 619. 1937.
7. Heller, C. G., H. Lauson and E. L. Severinghaus: *Am. J. Physiol.* 121: 364. 1938.
8. Marrian, G. F., and A. S. Parkes: *Proc. Royal Soc. London, s. B* 105: 248. 1929.
9. Szarka, A. J., and G. Kurtz: *Endocrinology* 23: 64. 1938.
10. Fluhmann, C. F.: *Endocrinology* 18: 705. 1934.

STUDIES ON THE RELATIONSHIP OF THE SEX HORMONES TO INFECTION II. THE EFFECT OF PSEUDOPREGNANCY ON THE SPREAD OF INDIA INK IN THE SKIN OF RABBITS

DOUGLAS H. SPRUNT AND SARA McDEARMAN

From the Department of Pathology, Duke University School of Medicine

DURHAM, NORTH CAROLINA

SEVERAL YEARS ago one of us (1) reported that pregnancy in rabbits altered the reactivity of the tissues to the virus of infectious myxomatosis. In the first paper of this series (2) we showed that the injection of estrone into castrated male rabbits decreased the spread of India ink in the skin and also increased the rabbits' resistance to infection with vaccinia.

These papers together with the literature indicate that variations in the sex hormones can cause modification in the susceptibility of the rabbit to infection. This fact seemed of sufficient importance to warrant further study. In the present experiment we have thought it desirable to learn more concerning the spread of materials in the normal animal before continuing the study of changes produced by the injection of the various hormones. Pseudopregnancy in the rabbit offered an excellent opportunity for this study, since it is not complicated by the presence of a fetus and a placenta. In this paper we are reporting results which show that pseudopregnancy decreases the spread of India ink in the skin.

In the preceding papers (1, 2) we reviewed much of the literature dealing with the effect of the sex hormones on the spread of tumors and infections in the tissues. At this time we will discuss only those papers not mentioned before. Ziskin and associates (3, 4) report that pregnancy and the estrogenic hormones decrease the amount of gingival infection. He attributes this decrease to a change in the mucosa of the mouth. Huberman and Israeloff (5) showed that the estrogenic hormones aid in the cure of gonococcal vaginitis, the improvement being attributed to the change in the nature of the mucosal surface. Young and Marks (6) advise the injection of amniotic fluid in the abdominal cavity prior to operation as a stimulant to a non-specific reaction.

METHODS AND MATERIALS

Animals. Only healthy adult female rabbits weighing about 2 kg. were used.

India ink. This was used in a dilution of 1 part India ink to 5 parts Locke's solution which was the same dilution as used by Hoffman and Duran-Reynals (7). The ink was injected intradermally in 0.5 cc. amounts through a 25-gauge needle. The injections were all made in the sides of the rabbit. Many of the injections were made

TABLE 1. THE SPREAD OF INDIA INK IN CONTROL RABBITS

Rabbit	Immediate sq. cm.	1 hr. sq. cm.	4 hr. sq. cm.	24 hr. sq. cm.
1	4.12	6.32	7.55	7.16
2	3.16	5.42	7.29	7.68
3	3.16	6.06	9.16	11.00
4	3.06	6.26	7.45	6.97
5	3.00	4.97	7.46	8.39
6	3.16	6.51	8.39	11.00
7	4.71	7.61	11.67	12.00
8	3.35	6.32	10.00	8.51
9	3.55	9.23	10.50	9.68
10	4.06	5.80	6.51	8.52
11	4.32	7.29	8.96	9.53
12	2.71	7.61	10.45	9.09
13	4.71	6.77	8.90	9.23
14	2.36	5.38	6.74	6.39
15	3.70	6.78	9.49	11.00
16	2.71	9.35	5.86	8.06
17	3.42	8.51	7.93	8.71
18	3.23	7.49	7.93	7.93
19	2.97	5.68	5.35	7.22
20	3.22	9.55	9.05	8.84
21	3.55	6.85	9.62	7.35
22	2.97	10.31	8.58	7.93
23	2.90	6.51	10.31	10.98
24	2.52	5.74	9.09	6.45
25	2.97	7.29	7.75	9.35
26	3.35	6.13	7.24	7.04
27	3.22	6.38	7.16	8.32
28	3.16	6.77	6.65	8.39
29	3.35	6.85	7.41	6.71
30	3.22	5.03	5.42	5.10
31	2.71	5.15	4.51	4.58
32	2.84	5.61	5.93	5.55
Mean	3.29	6.79	8.01	8.27
Mean Deviation	±0.38	±1.02	±1.44	±1.37
Standard Deviation	±0.56	±1.31	±1.70	±1.62
Standard Error of S.D.	±0.07	±0.17	±0.21	±0.20

TABLE 2. THE SPREAD OF INDIA INK IN PSEUDOPREGNANT RABBITS

Rabbit	Immediate sq. cm.	1 hr. sq. cm.	4 hr. sq. cm.	24 hr. sq. cm
1	2.90	5.61	6.06	4.97
2	3.03	5.80	5.28	4.51
3	2.97	5.80	5.10	4.71
4	2.84	4.90	5.28	4.97
5	2.97	6.58	4.71	6.58
6	3.28	4.97	6.71	5.15
7	2.45	5.15	6.85	5.15
8	2.45	5.03	7.41	5.61
9	2.26	4.45	6.45	6.20
10	3.22	5.15	7.49	5.80
11	2.58	4.77	9.09	6.97
12	4.32	5.35	5.48	7.06
13	2.58	8.51	7.35	5.80
14	2.71	6.38	8.39	6.77
Mean	2.89	5.60	6.54	5.73
Mean Deviation	±0.34	±0.76	±1.07	±0.72
Standard Deviation	±0.49	±0.99	±1.30	±0.82
Standard Error of S.D.	±0.09	±0.19	±0.24	±0.15

in duplicate, the mean of the 2 readings being taken. This was not continued throughout the experiment as it was found both in these rabbits and ones being used for other purposes that there were no significant differences between the 2 injections placed in the sides of the rabbits.

Production of pseudopregnancy. Some of the animals were stimulated by the intravenous injections of 200 U of APL gonadotropic hormone of pregnancy urine[1] and others by sterile coitus. As there were no essential differences between the 2 groups they are not separated. The ovaries contained large corpora lutea and the endometrium was of the pregnant type.

Control rabbits. These animals were females which had been isolated from males for at least 2 weeks. All were shown to have numerous Graafian follicles in the ovary but no corpora lutea. The endometrium of the uterus was of the non-pregnant type.

Measurement of areas. The spread of the India ink was traced immediately after injection and then at 1, 4, and 24 hours. The area of the spread was obtained with a planimeter.

Statistics. The usual methods were used for the calculation of the various statistical data. It should be mentioned, however, that 't' was derived from the formulas for small groups of material as given by Mainland (8).

Histological studies. The ovaries and the uterus were removed immediately after the 24-hour readings were made. Sections of these organs were prepared and stained with hematoxylin and eosin.

RESULTS

Tables 1 and 2 show clearly that pseudopregnancy reduces the spread of India ink in the skin. Statistical analysis of the data as shown in table 3 shows that there is a significant difference between the 2 groups in all the readings. The probability of the groups being random samples of the same group is highest in the immediate group. Here it is 3 in 100 but even this is lower than the 5 in 100 which is the usually accepted limit of significance. The available tables of 't' do not go beyond a probability of 0.01. This value was exceeded by the 1 and 4-hour groups and markedly so by the 24-hour one: hence, the difference in these groups is highly significant.

TABLE 3. STATISTICAL COMPARISON OF TABLES 1 AND 2

	Immediate	1 hour	4 hours	24 hours
t	2.20	2.84	2.77	5.43
P	0.03	<0.01	<0.01	<0.01

In examining the tables one notices that the significant deviation in the control group is higher than in the pseudopregnant group. Rabbits numbered 30, 31, and 32 account for much of this difference. It is thought likely, however, that there are factors other than hormones which will decrease the spread of India ink. One of these factors may have been present in these rabbits. The recent work of Joyner and Sabin (9) lends support to this idea. These workers showed that both tuberculosis and epizootic lymphadenitis decreased the spread of dye in the skin of guinea pigs.

[1] Kindly supplied by Parke, Davis & Co. as antuitrin-S.

DISCUSSION

The fact that pseudopregnancy in the rabbit inhibits the spread of India ink has important bearing on a number of phenomena. The work of Duran-Reynals (10) and our work (2) indicates that there is a close correlation between the spread of India ink and the spread of various disease incitants. The endocrine pattern in pseudopregnant rabbits is similar to that of most animals following ovulation and probably differs only slightly from that of pregnancy.

At present our data do not warrant any attempt to explain the mechanism by which the spread of particulate matter in the skin is inhibited. We do not believe that this change is purely a local one, although the local morphological changes used by Ziskin and associates (3, 4) and Huberman and Israeloff (5) to explain the beneficial effect of estrogenic substances in minimizing infections in the mouth and vagina undoubtedly play an important part. We think it likely that some other factor besides the inflammatory reaction plays a part in the beneficial effect of amniotic fluid (6) in laparotomies. This effect of the estrogenic and probably, also other hormones associated with pregnancy will, we believe, be found to be general, acting on all the tissues of the body. An indication that this is correct is that pregnancy affected the course of infectious myxomatosis both locally and throughout the body (1).

Numerous other instances of the effect of the endocrine pattern on infections such as the acne associated with menstruation could be cited, but we will postpone further discussion until more data are collected.

SUMMARY

In this report we have shown that the spread of India ink in the skin of 14 pseudopregnant rabbits was significantly less than the spread in 32 adult female rabbits.

The inhibiting effect of pseudopregnancy is discussed and similar examples of this are cited from the literature.

REFERENCES

1. Sprunt, D. H.: *J. Exper. Med.* 56: 601. 1932.
2. Sprunt, D. H., S. McDearman, and J. Raper: *J. Exper. Med.* 67: 159. 1938.
3. Ziskin, D. E.: *J. Dent. Research* 16: 367. 1937.
4. Ziskin, D. E., S. N. Blackberg, and C. A. Slanetz: *J. Dent. Research* 15: 407. 1936.
5. Huberman, J., and H. H. Israeloff: *J. A. M. A.* 103: 18. 1934.
6. Young, E. L., Jr., and G. A. Marks: *Surg., Gynec. & Obst.* 59: 610. 1934.
7. Hoffman, D. C., and F. Duran-Reynolds: *J. Exper. Med.* 53: 387. 1931.
8. Mainland, D.: The Treatment of Clinical and Laboratory Data, Oliver and Boyd, Edinburgh, 1938.
9. Joyner, A. L., and F. R. Sabin: *J. Epxer. Med.* 68: 325. 1938.
10. Duran-Reynals, F.; *J. Exper. Med.* 58: 451. 1933.

THE EFFECT OF TESTOSTERONE ON EXPERIMENTALLY PRODUCED ENDOMETRIAL HYPERPLASIA IN THE GUINEA PIG[1]

CLAIBORNE WILLIAMS, DORIS PHELPS
AND JOHN C. BURCH

From the Department of Obstetrics and Gynecology
Vanderbilt University School of Medicine

NASHVILLE, TENNESSEE

DURING the past several years the male sex hormone, testosterone, has been shown to have several definite effects when administered to the female (1–4). We have been most interested in the clinical reports of successful results obtained by the empirical use of testosterone in cases of functional uterine bleeding (5, 6, 7). The present study was undertaken in an effort to obtain information concerning the mechanism involved in the inhibition of bleeding which results from the administration of testosterone to women with functional menstrual disorders. In this study the effect of the simultaneous administration of theelin and testosterone upon the hyperplastic endometrium of the guinea pig has been investigated and the effects produced by this treatment have been compared to those produced upon the hyperplastic endometrium by testosterone alone, theelin alone, and theelin withdrawal.

METHODS

Thirty-five young adult castrate female guinea pigs were injected with 6.5 I.U. of theelin[2] for 40 days in order to produce endometrial hyperplasia (8, 9). Beginning immediately after this period of injections the animals were separated into 5 groups and treated as follows.

Group A. Ten received 6.5 I.U. theelin and 1 mg. testosterone[3] daily for 15 days.

Group B. Five received 6.5 I.U. of theelin daily for 15 days. These animals therefore received only theelin for a total of 55 days.

Group C. Five received 1 mg. of testosterone daily for 15 days.

Group D. Five received no further injections but were autopsied 15 days after the theelin injections were stopped.

Group E. Ten received no further treatment but were autopsied 24 hours after the last injection of theelin.

Five untreated female guinea pigs were autopsied 70 days after castration and used for control material.

The vaginal outlet of each animal was observed daily and smears were taken whenever the vagina was found open. All animals were autopsied 24 hours after

[1] This work has been aided by a grant to Vanderbilt University Schoool of Medicine from the Division of Medical Sciences of the Rockefeller Foundation.

[2] Parke, Davis & Company, theelin in oil, box marked No. 178, ampule numbered 3070694.

[3] Oreton (testosterone propionate in a solution of sesame oil), generously supplied by the Schering Corporation through the courtesy of Dr. Erwin Schwenk.

the last injection, except where otherwise specified. At autopsy the adrenals, pituitaries and thyroids were weighed in the fresh state. The uteri and vaginae were fixed in Bouin's solution and, after being sectioned, were stained with hematoxylin and eosin.

RESULTS

All animals which received long-term injections of theelin remained in constant estrus. The administration of testosterone to Groups *A* and *C* caused estrus to subside and the vagina to remain closed. Estrus also subsided in the animals in Group *D* following the withdrawal of theelin.

The endometria from the animals in Group *E* (theelin for 40 days) contained many moderate-sized dilated glands distributed throughout the stroma. The surface and gland epithelium was pseudostratified with tall cells, crowded nuclei and many mitotic figures. A few dilated glands showed cuboidal epithelium but the pseudostratified type was predominant. The nuclei were oblong and contained granular chromatin. The cytoplasm composed approximately two-fifths of the cells and was finely granular and uniformly stained. The surface epithelium showed moderate disintegration and separation from the endometrium. There were occasional small areas of mucification of the surface and glandular epithelium. The stroma was greatly thickened with moderate edema throughout. The stromal cells showed a strong proliferative reaction, with large oval, vesicular nuclei and many mitoses. Numerous capillaries were noted and many leucocytes were present, particularly near the surface. The vaginal epithelium was greatly thickened with many layers of cells supporting a prominent cornified surface. No leucocytes were present and there was no mucification.

The endometria from the animals in Group *B* (theelin 55 days) were comparable in all respects to those in Group *E*, except that the stroma was somewhat thicker, the glands somewhat more numerous, mucification a little more extensive, and leucocytes in the stroma slightly more numerous (fig. 1). The vaginal mucosa in this group showed essentially the same picture as was found in animals in Group *E*.

The endometria from animals in Group *D* (theelin withdrawal) showed a definite regression of proliferative changes in both epithelium and stroma (fig. 2). There were a few moderately large glands in the mid-portion of the stroma, and a moderate number of undilated glands deep in the stroma. The surface and gland epithelium showed only mild pseudostratification, little crowding of nuclei and a marked decrease in the number of mitotic figures as compared to Groups *E* and *B*. The epithelial cells were similar in appearance to those of Groups *E* and *B*, but the cytoplasm was much less abundant. There was essentially no separation or disintegration of the surface epithelium. The stroma was only slightly thicker than that found in the castrate controls, and contained little or no edema. The nuclei of the stromal cells were spindle and oval in shape and contained only occasional mitotic figures. A few capillaries were noted, but practically no leucocytes were seen. The vaginal mucosa was only 3 or 4 cells thick and contained no leucocytes, muci-

fication or cornification. This vaginal picture was comparable to that found in the castrate controls.

Endometria from the animals in Group C (theelin followed by testosterone) (fig. 4) showed a picture comparable in general to that seen in castrate animals injected with testosterone only for 34 days (10) (fig. 5). There were many dilated cystic glands near the surface and a moderate number of undilated glands deep in the stroma. In general, the surface and gland epithelium showed mild proliferative changes with mild pseudostratification in

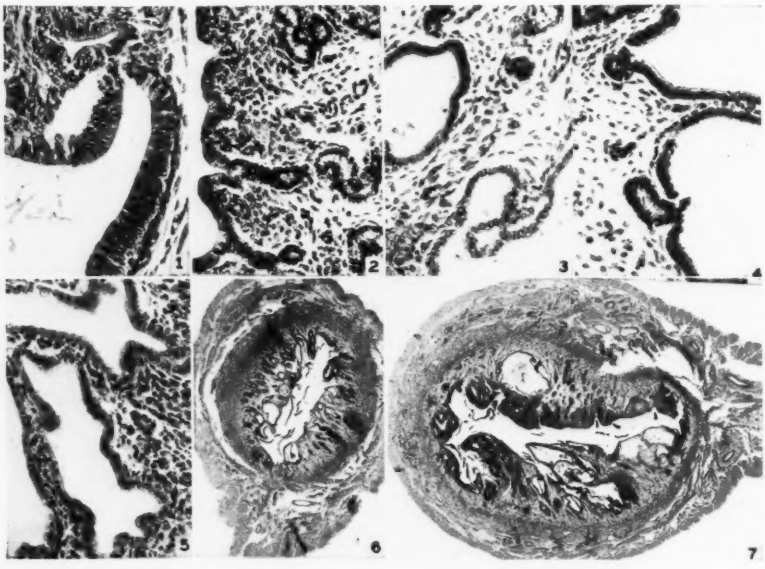


Fig. 1. ENDOMETRIUM FROM ANIMAL IN GROUP *B* (theelin 55 days) (× 125). Fig. 2. ENDOMETRIUM FROM ANIMAL IN GROUP *D* (theelin withdrawal) (× 125). Fig. 3. ENDOMETRIUM FROM ANIMAL IN GROUP *A* (theelin plus testosterone) (× 125). Fig. 4. ENDOMETRIUM FROM ANIMAL IN GROUP C (testosterone following theelin) (× 125). Fig. 5. ENDOMETRIUM FROM CASTRATE GUINEA PIG WHICH RECEIVED 1 mg. OF TESTOSTERONE DAILY FOR 34 DAYS (× 125). Fig. 6. CROSS-SECTION OF UTERUS FROM ANIMAL IN GROUP *A* (theelin plus testosterone) (× 9). Fig. 7. CROSS-SECTION OF UTERUS FROM ANIMAL IN GROUP *B* (theelin 55 days) (× 9).

some areas, moderate crowding of the nuclei, and a few mitotic figures. The cells were moderately tall, with slender oval nuclei, and finely granular, uniformly stained cytoplasm. The epithelium of the undilated glands was low columnar or pseudostratified in type, while the cells lining the largest dilated glands were cuboidal. There was moderate separation and disintegration of the surface epithelium, with essentially no mucification. The stroma was somewhat thicker and a little more edematous than that seen in Group *D*. The stromal cells showed a slight proliferative reaction with occasional mitotic figures. Few capillaries were noted and practically no leucocytes were seen. The vaginal mucosa was 3 to 6 cells thick, in contrast to the very thin mucosa seen in the castrates and in Group *D*. There were a

few leucocytes and moderate mucification but no evidence of cornification.

The uteri from the animals in Group *A* (treated withtheelin and testosterone) were much smaller than those from Group *B* (fig. 6, 7). The endometria were much thinner than those seen in Group *B* and contained a moderate number of thin-walled dilated glands near the surface (fig. 3). In some of these glands the epithelium was disintegrating. There was a moderate number of undilated glands deep in the stroma. The surface epithelium was mildly pseudostratified, that of dilated glands was low cuboidal and that of the undilated glands was low columnar and mildly pseudostratified. The surface and glandular epithelium showed few mitotic figures. The cells, in general, were not as tall as those seen in Groups *E* and *B*, but had the same nucleo-cytoplasmic ratio. The nuclei were slender, oval and vesicular. The surface epithelium was almost completely separated from the endometrium and showed marked disintegration. The stroma was greatly decreased in thickness as compared with that seen in Group *B*, and contained little edema. The stromal cells showed evidence of only moderate proliferation, with few mitotic figures. There were scattered capillaries present but only occasional leucocytes were seen. The vaginal mucosa in this group was about as thick as that seen in Group *B*, with marked mucification and many leucocytes, but no cornification.

The uteri from the animals autopsied 70 days after castration showed the typical castrate condition; the thin endometrium showing no proliferative changes, dilated glands or evidence of disintegration. The vaginal mucosa was thin, with no leucocytes or cornification.

The average weights of the pituitaries, thyroids, and adrenals from the various groups are listed in table 1.

TABLE 1

Group	No. of animals	Average body weight	Average organ weights		
			Pituitary	Adrenals	Thyroids
		gm.	*mg.*	*mg.*	*mg.*
A	10	590	15.0	192—181	69.7
B	5	540	13.8	143—112	73.0
C	5	530	11.8	105—104	53.8
D	5	540	12.2	131—124	70.2
E	10	538	13.4	145—133	68.4
Untreated castrates	5	535	16.2	154—128	83.5

DISCUSSION

Each of the procedures carried out in the above described experiments caused modifications in the hyperplastic endometrium produced by treatment with theelin for 40 days. Theelin alone (Group *B*) intensified all of the hyperplastic changes (slightly). Theelin withdrawal produced a decrease in the degree of proliferation and a disappearance of dilated glands, edema, leucocytes, desquamation and disintegration. Testosterone alone produced a decrease in the degree of proliferation, changed the epithelium of dilated

glands from pseudostratified to cuboidal (the latter is probably one criterion of decreasing proliferation) and produced no essential change in desquamation and disintegration of the surface tissue. Interestingly enough, the picture resulting from this treatment was entirely similar to that produced in the castrate endometrium by long-term injection of testosterone alone (10). Theelin plus testosterone (Group *A*) produced a decrease in the degree of proliferation, disappearance of edema and leucocytes, changes in the gland epithelium from pseudostratified to cuboidal and produced an intensification of desquamation and disintegration which was greater than that produced by theelin alone (Group *E*). Thus the modifications produced by each of these procedures were different in some respects from those produced by each of the other procedures. The changes produced by theelin plus testosterone were comparable to those produced by testosterone alone with respect to proliferation, glandular epithelium, edema and leucocytes, but were not comparable to the changes produced by this or any of the other procedures with respect to desquamation and disintegration. The effects produced by this combination of hormones suggests that testosterone nullified the effects of theelin to some extent, but since these effects were not entirely comparable to those produced by testosterone alone or theelin withdrawal, they cannot be attributed solely to antagonism of theelin by testosterone. They must have resulted, rather, from the combined action of the two hormones. Each procedure produced modifications in the vaginal mucosa which were comparable to the changes produced in the endometria of the same group.

When testosterone is administered to the human female, with either normal or abnormal menstrual function, the endometrium is presumably subjected to the action of this substance in the presence of estrogen. In view of the fact that in either case this treatment results in the inhibition of bleeding, it is interesting that the effects of the simultaneous injection of estrogen and testosterone upon the hyperplastic endometrium of the guinea pig consist essentially of a decrease in proliferative activity, disappearance of edema and leucocytes (that is, change in influence on blood vessels), modification of the glandular epithelium from pseudostratified to cuboidal and desquamation and disintegration of the surface epithelium.

SUMMARY

The effects of theelin alone, testosterone alone, theelin plus testosterone, and theelin withdrawal upon experimentally produced endometrial hyperplasia in the guinea pig were compared. Each of these procedures produced certain definite modifications in the hyperplastic endometrium. Theelin alone caused a slight intensification of the proliferative reaction. Theelin plus testosterone produced decreased proliferation, a decrease in edema and leucocytes, thin-walled cystic glands and an increase in disintegration of surface tissue. These changes were comparable to those produced by testosterone alone, except for the intensification of disintegration. Neither the changes

produced by testosterone plus theelin nor those produced by testosterone alone were comparable to the changes produced by theelin withdrawal.

REFERENCES

1. Zuckerman, S.: *Lancet* 2: 676. 1937.
2. Hartman, C. G.: *Proc. Soc. Exper. Biol. & Med.* 37: 87. 1937.
3. Papanicolaou, G. N., H. S. Ripley and E. Shorr: *Endocrinology* 24: 339. 1939.
4. Mazer, M., and C. Mazer: *Endocrinology* 24: 175. 1939.
5. Hamblen, E. C.: *J. M. A. Georgia* 26: 368. 1937.
6. Ellison, E. T., and J. C. Burch: Unpublished observations.
7. Mazer, C., and M. Mazer: *Endocrinology* 24: 599. 1939.
8. Wolfe, J. M., M. Campbell and J. C. Burch: *Proc. Soc. Exper. Biol. & Med.* 29: 1263. 1932.
9. Simpson, J. W., and J. C. Burch: *Proc. Soc. Exper. Biol. & Med.* 32: 1570. 1935.
10. Phelps, D., E. T. Ellison and J. C. Burch: *Endocrinology* 23: 458. 1938.

EDITORIAL ARTICLE

INTERNATIONAL STANDARDS FOR PREGNANCY URINE GONADOTROPHIN, MARES' SERUM GONADOTROPHIN AND CERTAIN ANTERIOR PITUITARY HORMONES

THE REPORT OF THE THIRD INTERNATIONAL CONFERENCE ON THE STANDARDIZATION OF HORMONES recently published contains information in regard to setting-up and making generally available dry powder standards of the gonadotrophic substance of pregnancy urine (the so-called anterior pituitary-like gonadotrophic hormone), the gonadotrophic substance of pregnant mares' serum and certain of the anterior lobe principles. This is indeed a step foward and one which should be welcomed equally by all those concerned in the study of these hormones, either in the laboratory or in clinical practice, as well as by the various manufacturers of these biological products. The Department of Biological Standards of the National Institute for Medical Research, Hampstead, London, has very generously undertaken the task of preparing and distributing the standards after such time as the various materials which have been donated for use in the preparation of such standards have been assayed by many collaborating laboratories and general agreement has been reached as to the nature, strength, etc., of the final mixture which is to constitute the standard. Already the standard for the gonadotrophic substance of the urine of pregnancy has been prepared and distributed and it may be confidently expected that standards for the gonadotrophic substance of pregnant mares' serum and for prolactin will be made available very soon.

PREGNANCY URINE GONADOTROPHIN

The League of Nations Health Organization memorandum on the international standard for the gonadotrophic substance of human urine of pregnancy (chorionic gonadotrophin), issued with the sample of the standard powder, is very informative. Excerpts from this are quoted below.

"During 1937 and 1938 an enquiry was made among interested workers as to the practicability and desirability of establishing an International Standard for the gonadotrophic substance of human urine of pregnancy. It was recalled that preparations of this substance were standardised in a great variety of ways, and that there was no method of equating the various types of units, defined in terms of animal reactions, used by different workers and manufacturing organizations. The survey showed that there was complete unanimity as to the desirability of establishing a standard, and the offers of material received from various quarters made it evident

that there would be no practical difficulty in constituting a standard preparation in adequate quantity to serve world requirements.

At the Third International Conference on the Standardisation of Hormones held in Geneva in August, 1938, under the auspices of the Permanent Commission on Biological Standardisation of the Health Organization of the League of Nations, the results of the preliminary survey were considered, and it was unanimously decided to arrange for the establishment of an International Standard for the gonadotrophic substance of human urine of pregnancy.

The Conference had before it the results of a co-operative examination of the various contributions offered towards the proposed standard, and they were able to formulate details of the manner in which the contributions should be mixed. They also defined a unit of activity in terms of the proposed mixture, subject to confirmation after specimens of the mixture had been assayed by members of the Conference.

The Contributions were subsequently mixed as arranged and a specimen approved by each member of the Conference. The Standard was established, and the unit brought into current use, on 1st, April 1939.

Definition of the unit. The definition of the unit adopted by the Third International Conference is as follows.

> The specific gonadotrophic activity of 0.1 mg. (100γ) of the standard preparation shall be the International Unit for recording the activities of all gonadotrophic preparations of human urine of pregnancy, *but only of such.*

The unit so defined is an amount of activity very similar to that required, under the conditions used by many workers, to cause cornification of the vaginal epithelium of the immature rat.

Suggestions for use. After the ampoule has been opened, the inner tube should be removed, freed from cotton wool and closed with the cork provided. One or more tablets, each of which contains *about* 100 units, should be removed as required. The tablets are as constant in size as is practicably possible, but it is essential that they should be weighed before use, so that a solution can be made of an exactly known strength, on the basis of 10 units for each milligramme of ascertained weight. The tablets dissolve very readily in water or saline.

After the ampoule has been opened, the amber tube containing the tablets of the standard preparation should be kept in a small desiccator containing P_2O_5.

Use of standard in biological assay. The following recommendations for use of the standard are taken from the Report of the Conference, Bulletin of the Health Organization of the League of Nations, 7: 889, 1938.

> 'The only tests for the comparative determination of gonadotrophic activity, in units as above defined, on which the Conference has evidence to justify recommendation, depend on:
>
> (*a*) The observation of a direct or indirect gonadotrophic effect, shown by morphological changes in the gonads;
>
> (*b*) The observation of secondary changes in the accessory reproductive organs, in animals not deprived of their gonads. When this type of test is used, the absence of substances directly causing such changes in the accessory reproductive organs should be assured by control tests on animals deprived of their gonads.' "

The chief advantage to be gained by a general adoption of the international unit for preparation of chorionic gonadotrophin will be in the establishment of uniformity of all products so standardized. Theoretically it is immaterial what type of biological response is made use of in individual

assays, provided always that a direct comparison is made between the product being assayed and the international standard.

Sir Henry Dale named, among others, the following principles which should be fulfilled in the standardization of a hormone or drug by a biological method:

> "(1) Measurements made by biological means, like every other kind of measurement, must be essentially comparative. No assay has any serious value, unless it is made with reference to an accepted standard.
> (2) The standard chosen for the comparative test should be, or should owe its activity to, the active principle for which the preparation is to be assayed; and the active principle in question should be that to which the therapeutic value is due.
> (3) If the test measures the important active principle, the biological reaction employed need have no relation to the therapeutic effect."

In the standardization of pregnancy urine gonadotrophin in terms of international units by one of the accepted biological methods no principle of Dale is violated.

The actual method used with which to compare the unknown with the international standard powder will no doubt depend to a large extent on the preference of the individual worker. The production of vaginal estrus in a definite percentage of a large group of 21-day-old female rats has commended itself to several workers, including the writer of this editorial, as an extremely satisfactory method of assay.

GONADOTROPHIN OF PREGNANT MARES' SERUM

It has been agreed that it is desirable and practicable to establish an international standard preparation for the gonadotrophic substance of the serum of the pregnant mare. Several contributions which have been donated for use in the preparation of the standard have been assayed by a number of cooperating laboratories and in the light of the data thus obtained the international standard powder is now being prepared. This is being done by combining the various preparations which have been accepted and mixing therewith sufficient inert diluent. It was further agreed that the international standard preparation so prepared should be accepted as the standard for the assay of all preparations of mares' serum gonadotrophin, but only of such. The same methods of preparation of tablets, of packing and of refrigeration, etc., which were used in the case of the urinary gonadotrophin standard are to be used here.

THYROTROPHIC SUBSTANCE OF THE ANTERIOR PITUITARY

The Conference has recommended the establishment of an international standard for the thyrotrophic substance of the anterior pituitary gland, and also one for the lactogenic substance (crop-gland-stimulating substance, prolactin). The report of the Conference in so far as it concerns these is herewith quoted.

"*Consideration of the thyrotrophic substance of the anterior lobe of the pituitary gland.* It was agreed:

> (1) That this Conference agrees with the opinion expressed in the replies to the questionnaire circulated last year that it is both desirable and practicable

to establish an international standard preparation for the thyrotrophic substance of the anterior lobe of the pituitary gland.

The Conference then discussed the possibility of obtaining preparations of the thyrotrophic substance of the anterior lober of the pituitary gland free from contamination by other anterior lobe principles, in particular the gonadotrophic substance, and from active constituents of the pars intermedia and the posterior lobe. It was then further agreed:

(2) That the materials offered by several manufacturing and other organisations should be circulated among certain experts, including some members of the Conference, in order that they might be tested as to their suitability as constituents of a mixed preparation of the thyrotrophic substance in the form of a dry powder, to serve as the international standard preparation. The opinion was expressed that it would be desirable, if it were found practicable, to obtain all the contributions in the form of material prepared from the dissected anterior lobes of the pituitary glands of oxen only. In order to facilitate comparative testing of the different samples available, it was decided that one of these (referred to as '*TH 5*' in document *MM5*) should be used as a provisional standard of reference for the others.

(3) That the specific thyrotrophic activity of 0.25 milligramme (250γ) of the preparation *TH5* would afford a suitable basis for the definition of an equivalent unit, for acceptance by international agreement, in terms of the standard preparation, as ultimately made ready for distribution.

(4) That the Chairman and Secretary should undertake to get some trial tablets made of the standard powder, when available, with the addition, if necessary, of a suitable base to give the material the required consistency.

(5) That when these different trials were completed, and the definition of the unit in terms of this standard material had thus been confirmed and finally accepted, after examination in its completed form by the experts co-operating in the tests, arrangements should be made for the compression of the standard material into tablets, each containing a suitable number, such as 50, of the agreed units, and for the packing of these tablets in suitable numbers, after complete desiccation, in sealed glass containers, under conditions excluding oxygen.

(6) That the standard shall thereafter be kept at a temperature below 0° C., and protected from light, at the National Institute for Medical Research, Hampstead, London, England, and distributed therefrom on behalf of the League of Nations Health Organization to national centres for the control of such substances, and to other suitable research institutes.

The Conference then discussed the nature of the available biological tests for thyrotrophic activity, particularly with regard to the necessity of excluding effects due to non-specific active constituents, either of the standard preparation or of other preparations assayed in comparison therewith. It was then further agreed:

(7) That the only tests available for such comparative determination of thyrotrophic activity, which were free from this danger of interference by non-specific constituents, were tests based on the direct observation of changes induced in the thyroid gland itself, and that, in particular, indirect tests, based upon effects on metabolic rate, were not trustworthy when applied to such incompletely purified materials as are yet available.

(8) That each member of the Conference participating in the confirmatory tests on the standard material be requested to furnish, with his report, a detailed description of the method or methods of biological comparison used in his tests,

with a view to the publication of an annex to the report of the Conference, dealing with methods of assay.

(9) That it is desirable that researches should be continued in appropriate centres, with the object of discovering methods for separating the thyrotrophic hormone more completely from other active principles of the pituitary gland, on a scale sufficient to enable the standard preparation, now adopted, eventually to be replaced by a new standard preparation, free from such other active principles.

Consideration of the lactogenic (crop-gland-stimulating) substance of the anterior lobe of the pituitary gland ("*prolactin*," "*galactin*," "*mammotropin*"). It was agreed:

(1) That this Conference agrees with the opinion expressed in the majority of the replies to the questionnaire circulated last year that it is both desirable and practicable to establish an international standard preparation for the lactogenic (crop-gland-stimulating) substance of the anterior lobe of the pituitary gland.

(2) That the preparations of this principle offered by several manufacturing and other organisations should be circulated among certain experts, including some members of the Conference, in order that they might be tested as to their suitability as constituents of a mixed preparation of this principle in the form of a dry powder, to serve as the international standard preparation.

(3) That no contribution offered towards this standard preparation should be accepted, if it is found, on examination, to contain less than about 5 'Riddle-Bates units' of the specific activity in 1 milligram.

(4) That the preparation made by careful mixture of the acceptable contributions shall constitute the international standard preparation without dilution.

(5) That this standard preparation shall be assayed by the co-operating experts in 'Riddle-Bates units,' in comparison with the preparation named '*L.P.1*' in the table on page 4 of document *MM5*.

(6) That by comparison of the assays thus carried out, agreement shall be reached among the members of the Conference as to the activity of the standard preparation in 'Riddle-Bates units' per milligramme, and that the international unit shall be thus defined in terms of the standard preparation.

The Conference then proceeded to discuss the possibility of obtaining a greater and more uniform activity among the different samples contributed for the preparation of the standard, by attempting to separate the active constituent in crystalline form, but concluded that, on the information before the Conference, such an attempt would be unlikely, under present conditions, to have an effect in the desired direction It was then further agreed:

(7) That the Standards Department of the National Institute for Medical Research be asked to consider the practicability of issuing this standard in two forms, one without dilution, the other suitably diluted with an inert substance and compressed into tablets of convenient dimensions, in order to effect economy in the use of the standard in tests requiring the use of small fractions of a unit.

(8) That, when the definition of the unit has thus been confirmed and finally accepted, the standard material shall be distributed into suitable glass containers, in the form of the untreated powder, and possibily also after dilution and compression into tablets as above suggested, and that the contents of such containers shall then be completely desiccated, and the containers sealed under conditions as nearly as possible approaching sterility and excluding oxygen.

(9) That the standard shall thereafter be kept at a temperature below 0° C., and protected from light, at the National Institute for Medical Research, Hampstead, London, England, and distributed therefrom, on behalf of the League of Nations Health Organisation, to national centers for the control of such substances, and to other suitable research institutes.

(10) That the Conference cannot, on the information before it, recognise any test as giving sufficiently quantitative results for the comparative assay of preparations in relation to this standard, except those which depend on the measurement or observation of growth produced in the crop gland of the pigeon or dove, whether as the result of systemic or of local administration.

(11) That each member of the Conference participating in the confirmatory tests on the standard material be requested to furnish, with his report, a detailed description of the method or methods of biological comparison used in his tests, with a view to the publication of an annex to the report of the Conference, dealing with methods of assay."

GONADOTROPHIC, CORTICOTROPHIC AND GROWTH-PROMOTING PRINCIPLES OF THE ANTERIOR PITUITARY

The Conference did not feel that the time was yet ripe for the setting-up of standards for the gonadotrophic, corticotrophic or growth-promoting principles of the anterior pituitary gland. The report concerning this was as follows.

"It was agreed that, on the information before it, the Conference is unable to recommend the establishment of a standard preparation for measurement of units of the gonadotrophic effects of preparations from the anterior lobe of the pituitary gland. It has before it the evidence that, in certain laboratories, preparations have been obtained in which different factors of the total gonadotrophic activity are more or less completely separated. In these circumstances, the Conference believes that future action, in the direction of establishing a standard or standards for the principle or principles in question, can best be accelerated by arranging for the exchange of specimens of the preparations claiming to present the separated activities, among the experts concerned and others interested, for co-operative trials and interchange of experience.

Consideration of the corticotrophic (adrenotrophic) substance of the anterior lobe of of the pituitary gland. It was agreed:

(1) That it is desirable to establish an international standard for this substance, but that, on the evidence as yet available, it does not seem to be practicable to collect sufficiently suitably purified material to constitute a standard preparation, on the scale which would be necessary to make it effectively available for international distribution.

(2) That, in these circumstances, the policy most likely to accelerate action in the direction of establishing a standard would be the exchange of specimens among the experts concerned and others interested in co-operative trials.

Consideration of the growth-promoting hormone of the anterior lobe of the pituitary gland. The Conference, after full discussion, accepted the following resolution by unanimous vote.

That the members of the Conference recognize that some of the objections which might be urged against the establishment of a standard for the growth hormone could be urged against some of the standards, for other forms of an-

terior pituitary activity, which they have accepted; that they are nevertheless of the opinion that the only action on which agreement can be reached, on this occasion, with regard to the growth-promoting hormone, is the arrangement of a co-operative investigation, with a view to the establishment of a standard for growth-promoting activity at the earliest date at which the basis for agreed action appears to be available."

NOMENCLATURE

The Conference gave some attention to the question of nomenclature and the following statement concerning this appears in the report. It was agreed:

"(1) That the fully descriptive term 'anterior lobe of the pituitary gland' should be adopted for reference to the organ in question in the records of the Conference.

No general agreement was reached in discussion of the respective claims of the suffixes 'trophic' and 'tropic' in the construction of descriptive terms for reference to the active principles with which the Conference had dealt. It was agreed:

(2) That the use of the forms with the suffix 'trophic' or 'trophique' in the official English and French versions of the discussions and decisions of the Conference would be acceptable to the members of the Conference. It was agreed further that the form 'tropic' might, if necessary, be used with appropriate modifications in translations into other languages.

(3) That the Conference recommend the adoption, for general scientific use, of the term 'gonadotrop(h)in' for reference to principles with gonadotrophic action, with the addition, in each case, of a suitable adjective or descriptive phrase, to indicate the source of origin of the particular principle to which reference is made.

(4) That the Conference recommend the adoption, for general scientific use, of the terms 'corticotrop(h)in' and 'thyrotrop(h)in,' to denote the active principles of the anterior lobe of the pituitary gland acting on the adrenal cortex and on the thyroid gland respectively."

It is apparent from the above quotations that very definite progress is being made toward the establishment and distribution of international standard preparations of the various gonadotrophic hormones and of anterior pituitary principles. Although as yet only the standard of chorionic gonadotrophin is available, the writer has been informed that the standard for gonadotrophin of pregnant mares' serum will be ready for distribution within a few weeks, and that for prolactin shortly thereafter. It is to be hoped that as these standards become available all workers will avail themselves of them.

J. B. COLLIP

DEPARTMENT OF BIOCHEMISTRY AND PATHOLOGICAL CHEMISTRY
MCGILL UNIVERSITY, MONTREAL, CANADA

CLINICAL AND LABORATORY NOTES

THE EFFECT OF ESTROGEN ON THE SKELETAL AGE OF IMMATURE RATS

NATHAN B. TALBOT*

PEDIATRICIANS are familiar with the fact that the skeletal age of sexually precocious children is frequently advanced beyond the normal for their chronological age. This fact is determined by comparing the number, size and shape of the centers of ossification and the bones of the extremities with normal standards. It has been suggested that the sex hormones may be responsible for the precocious bone development. There is, however, little experimental evidence to support this thesis. The present investigation was undertaken in an attempt to determine whether estrogen has any influence on the rate at which the skeletons of immature rats develop.

Spark and Dawson[1] have described the order and time of appearance of the centers of ossification in the fore and hind limbs of the albino rat. Their data show that the number of centers increases with age in a reasonably consistent manner during the first month of life. This increase corresponds for the rat to that described by Todd[2] for the child during the first 13 to 15 years of life. The effect of estrogen on the skeletal age of the rat may, therefore, throw some light on the problem presented by the precocious child.

Nine litters comprising a total of 44 albino rats were used in this experiment. Of these 29 were females and 15 were males. At least half of each litter were kept as controls. Only litter mates of the same sex were compared because, as Spark and Dawson have shown, females tend on the average to have more centers of ossification than the males of the same age. Beginning on the first day of life 2 or 3 out of a litter of 5 or 6 rats were given daily injections of 12.5 micrograms estradiol in 0.1 cc. sesame oil under the skin of the back. The remainder served as normal controls. All the animals were suckled by the mother until the 8th or 10th day of life when they were killed with chloroform. Frequent weighings during life disclosed no significant difference in the weight of control and treated litter mates. Autopsy showed that the uteri of the control rats were within normal limits for age, whereas the uteri of treated animals were precociously enlarged. This shows that the control rats did not receive significant amounts of estrin by contact. No differences between the sex organs of treated and control males was discerned. The number and relative size of the ossification centers of the hind and fore limbs were noted after they had been cleared and stained according to Dawson's methods.[3]

[1] From the Biological Laboratories, Harvard University, and the Department of Pediatrics, Harvard Medical School, Boston, Mass.

[1] SPARK, C., AND DAWSON, A. B.: *Am. J. Anat.* 43: 411. 1928.

[2] TODD, T. W.: The Atlas of Skeletal Maturation. Mosby, St. Louis, 1937.

[3] DAWSON, A. B.: *Stain Technology* 1: 123. 1927; *Science* 64: 378. 1927.

The data obtained (table 1) show that estrogen causes a distinct increase in the size and number of ossification centers in the females, but not in the males of the same age. Treated females have an average of 32% more centers than control females, whereas the mean difference between the treated and control males is 2%. Incidentally, while there is no significant variation in the average size and number of osseous centers of control litter mates, there is a difference between the control rats

TABLE 1. THE EFFECT OF ESTRADIOL ON THE DEVELOPMENT OF THE OSSIFICATION CENTERS OF IMMATURE RATS

Litter no.	No. of rats	Age, days	Average number of ossification centers per rat									Relative size	Av. total no. centers per rat. fore limb	% increase fore limb
			Hind limb					Fore limb						
			A[1]	B	C	D	E	F	G	H	I			
Females														
7 Ex[2]	2	8	6	1	1	4	3.5	9	4	4	4	+++	21.0	22
C	3	8	5	1	1	3	0.5	8	4	4	1.3		17.3	
2 Ex	2	8	2	0	1	0	0	5.4	2.7	0	0	+	8.1	6
C	3	8	2	0	1	0	0	5	2.3	0	0		7.3	
3 Ex	2	10	5	1	1	0	0	9	4	4	2.5	+++	19.5	58
C	3	10	4.3	0	1	0	0	7	4	1.5	0		12.5	
4 Ex	2	10	5	0.5	1	0	0	9	4	4	4	+++	21.0	35
C	3	10	5	0	1	0	0	8	4	3.7	0		15.7	
5 Ex	2	10	5	1	1	0	0	9	4	4	1	+++	18.0	41
C	3	10	5	0	1	0	0	8	4	0.7	0		12.7	
6 Ex	2	10	5	1	1	0	0	9	4	4	2	+++	19.0	31
C	2	10	5	0	1	0	0	7.5	4	3	0		14.5	
Av. Ex	12											+++	17.8	32
Av. C	17												13.3	
Males														
7 Ex	2	8	2	0	1	0	0	5	3	0.5	0	0	8.5	6
C	2	8	2	0	1	0	0	5	3	0	0		8.0	
8 Ex	3	8	2	0	1	0	0	4.3	0	0	0	0	4.3	(− 2)
C	3	8	2.3	0	0.7	0	0	3.7	0.7	0	0		4.4	
9 Ex	2	10	3.3	0	1	0	0	7	4	2	0	0	13.0	2
C	3	10	3.7	0	1	0	0	6	4	1	0		11.0	
Av. Ex	7											0	8.6	2
Av. C	8												7.8	

[1] The capital letters refer to the following ossification centers: A, Tarsals; B, Distal Fibula; C, Distal Tibia; D, Distal Metatarsals; E, Proximal First Phalanges (hind limb); F, Carpals; G, Distal Metacarpals; H, Proximal First Phalanges (fore limb); I, Middle Phalanges (fore limb).

[2] Ex refers to the rats which received estradiol: C refers to the control rats.

of one litter and those of another litter of the same age. Therefore, it is important to use litter mates as controls. Figure 1 shows the magnitude of the difference in size of osseous centers of two control and treated female litter mates at 10 days of age. Equally distinct differences in size are present in all the centers of ossification in the females but not in the males. The average increase in the number and size of centers observed in the females corresponds to an advance in the skeletal age of the treated rats of between one and two days over the normal controls.

Gardner and Pfeiffer[4] have shown that estrogen causes hyperossification of the

[4] GARDNER, W. U., AND C. A. PFEIFFER: *Proc. Soc. Exper. Biol. & Med.* 37: 678. 1937.

femurs of mice. It is probable that the advanced osseous development observed in the present series of female rats is a similar phenomenon. It is not clear, however, why the females reacted whereas the males did not. One possible explanation is suggested by Gardner and Pfeiffer's[5] recent work in which they showed that male mice did not respond to estrogen as rapidly as female mice and that testosterone inhibited the action of estrin on bones.

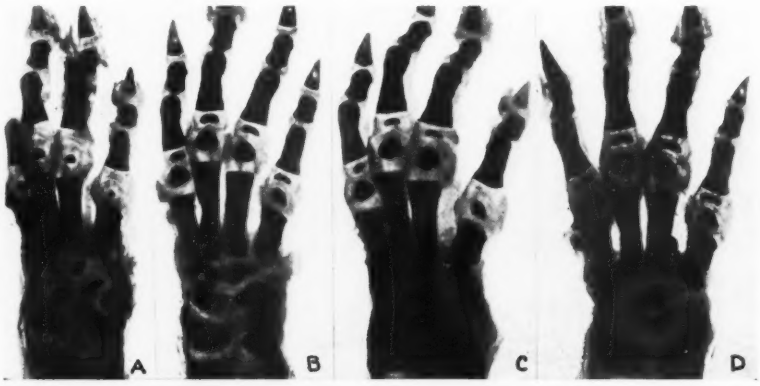


Fig. 1. Effect of estradiol on the size of distal metacarpal and proximal first phalangeal ossification centers (fore limb). A, Control, Litter No. 3; B, Estrin Treated, Litter No. 3; C, Control, Litter No. 4; D, Estrin Treated, Litter No. 4.

The findings reported above are interpreted to mean that estrogen may be one of the factors which is responsible for the advanced osseous development noted in precocious puberty. It is probable, however, that other factors are important.

Summary. Estradiol administered to immature rats for from 7 to 9 days after birth causes a significant advance in the skeletal age of the females, but not of the males.

[5] Gardner, W. U., and C. A. Pfeiffer: *Anat. Rec.* Vol. 73, #3 and Supplement #2, March, 1939, p. 21.

THE EFFECTS OF ANDROGENS UPON THE FISH (*LEBISTES RETICULATUS*)*

WILBURN J. EVERSOLE†

THE RÔLE of sex hormones in early sexual differentiation, including the development of the gonad, is being intensively studied in many forms, including fish.[1,2,3] The guppy, *Lebistes reticulatus*, is a convenient fish in which to make such studies. Berkowitz[1] found that estrogens suppressed male and induced female secondary characters in male guppies if treatment began before sexual maturity; withdrawal of hormone treatment then permitted male characters to appear coexistent with induced female characters. He found, further, that estrogens had little if any effect upon the ovary, but in a high percentage of cases induced either a suppressed or hermaphroditic type of male gonad when given to males.

This study is complementary to that of Berkowitz and is concerned with the effects of the androgen, testosterone propionate,‡ upon the above-mentioned phenomena. The results indicate, as in birds, that different types of control influence different secondary sexual characteristics and that the whole subject is one of great complexity.

A total of 100 fish were treated with testosterone propionate. Fish were kept in 2½ gallon aquaria, in groups of from 4 (if adults) to 16 (young broods). The temperature of the water was thermostatically controlled at approximately 78° F. Two-thirds of the guppies were injected pleuroperitoneally twice weekly, by means of a microinjection apparatus, with approximately 0.03 mg. per injection of testosterone propionate over a period ranging from one week to 4 months. At the initial injection these fish were somewhat less than 2 months old and secondary sex characters were indistinguishable. The non-effect of sesame oil, the solvent of testosterone propionate, was determined by injecting this substance into 18 animals for a period varying from 2 to 3 months. In one-third of the cases the hormone was fed, by adding it in ether solution to the food (Reliance) and then evaporating the mass to dryness. Feeding from birth was done in such amounts that one half mg. of hormone was added daily to each aquarium for periods varying from 2 to 3½ months. The fish responded more readily and completely to testosterone injections than they did to hormone feeding. Thirty-four litter mate controls were raised and handled in a manner identical with the experimental animals except that hormone administration was not employed.

Female fish fed or injected with the hormone readily assumed some but not all of the male secondary sex characters (fig. 1B). Estrogens, on the contrary, induced a full complement of female secondary sex characters in males.[1]

In males the anal fin develops into a gonopod. The control of this trait is apparently entirely dependent upon the male sex hormone, since it can be produced in

* This work was submitted in partial fulfillment of the requirements for the degree of Master of Science in New York University.

The author is grateful to Dr. Robert Gaunt who suggested this work and to Mr. Philip Berkowitz for advice as to the proper care of animals.

† From the Department of Biology, Washington Square College, New York University, New York City.

[1] BERKOWITZ, P.: *Anat. Rec.* 71: 161. 1938.

[2] PADOA, E.: *Monit. Zool. Ital.* 48: 195. 1937.

[3] WITSCHI, E., AND E. N. CROWN: *Anat. Rec.* 70: 121. 1937.

‡ Crystalline testosterone propionate was kindly supplied by Dr. Max Gilbert of the Schering Corporation, Bloomfield, N. J.; and testosterone propionate in oil (perandren) by the Ciba Company.

females at any stage of development by testosterone administration. The cause of its suppression in males by estrogenic substances, as found by Berkowitz, is not clear. It may have been due to the estrogenic inhibition of the male gonad.

Testosterone does not induce in females the typical male red, yellow and orange body pigment, although estrogens given before sexual maturity will prevent their appearance in males.[1] The control of these traits is probably not purely hormonic.

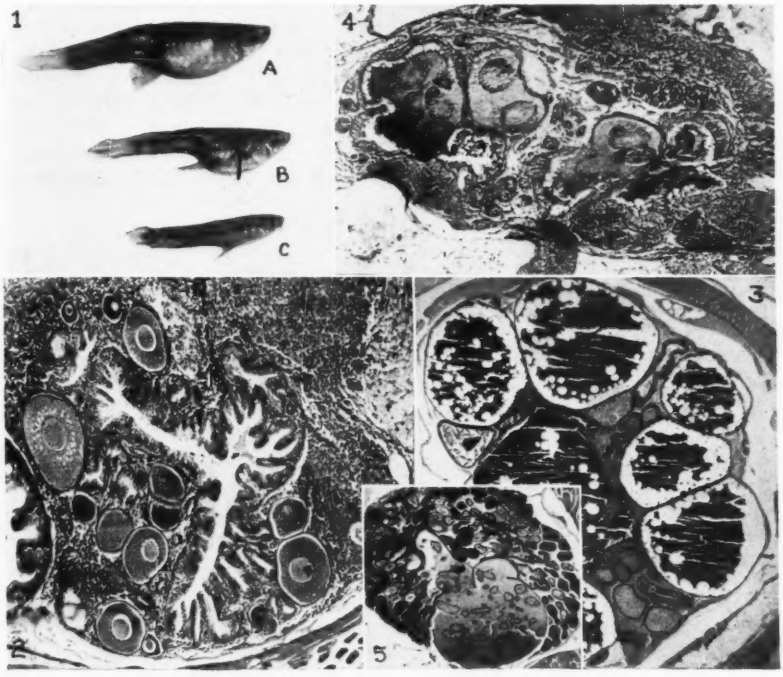


Fig. 1A. Normal mature female. ×1.2. Fig. 1B. Female treated from birth with testosterone propionate. Note the induced gonopod, induced black spot (shown by arrow) and small size. ×1.2. Compare with fig. 1A and 1C. Fig. 1C. Normal mature male. ×1.2. Fig. 2. Cross section of an inhibited ovary from a female which had been injected biweekly with testosterone propionate for a period of 3.5 months. Note the small size, absence of well-developed yolk eggs, and lymphoid-like tissue. ×72. Compare with fig. 3. Fig. 3. Cross section of a normal ovary with large yolk eggs from a normal mature female. ×16. Fig. 4. Cross section of an inhibited testis from a male which had been injected biweekly with testosterone propionate for a period of 3.5 months. Note the marked degeneration, inhibited gametogenesis, and secondary invasion of lymphoid-like tissue. ×72. Compare with fig. 5. Fig. 5. Testis of a normal male showing many spermatophores. ×16.

The appearance of typical male black spots behind the pectoral fins in almost all of the testosterone-injected females indicates that male sex hormone is necessary for the expression of this characteristic in both sexes.

In a still different category is the control of the gravid spot, a distinctive female trait. It can be brought out in males with estrogens,[1] but we were unable to suppress it in females with testosterone.

A curious effect of male hormone, since no such action occurs in mammals,[4] is its suppression of growth in females. Mature females are approximately 30 mm. in

[4] McEuen, C. S., H. Selye and J. B. Collip: *Proc. Soc. Exper. Biol. & Med.* 36: 390. 1937.

length and mature males 18 mm. Testosterone suppressed the growth of females to such an extent that they never exceeded a length of 21 mm. and small, oil-injected, controls overcame and surpassed, in size, the hormone-treated animals. In normal development, similarly, growth of males stops abruptly at the time of testis maturity. Estrogens stimulate the growth of males to an approximate female size,[1] perhaps by suppressing testis activity.

Testosterone treatment does not accentuate or alter the expression of the secondary sex characters of the male.

Effects on gonads. Testosterone treatment in sexually mature females uniformly inhibited gametogenic activity, causing a resorption of yolk and of embryos, if present. In more than 50% of these females, the ovaries, besides being markedly reduced in size, were partially or completely surrounded by an atypical mass of lymphoid appearing tissue (fig. 2). In only 3 cases were definite ovotestes (obviously transformed ovaries) observed. This is an insignificant number in view of the occasional spontaneous occurrence of such gonads.[5] Testosterone evidently lacks the sex-reversing potency of the estrogens since the latter substances, in over one-half of the cases, induced the development of ovotestes in males.[1] In this respect the guppy responds to crystalline sex hormones in a fashion somewhat similar to Amblystoma.[6,7]

The male gonad was apparently unaffected by treatment of short duration. However, males receiving injections exceeding a period of 3 months showed indications of inhibited spermatogenesis and a secondary invasion of lymphoid appearing tissue (fig. 4).

Summary. Administration of testosterone propionate to the female guppy suppressed body growth and ovogenesis, and induced the development of some but not all of the male secondary sex characters.

It is apparent that even in these lower vertebrates the control of sexually differentiating traits is a complicated one in which genetic or intracellular and endocrine factors are probably intermixed.

---

[5] Blacher, L.: *Biol. Bull.* 50: 374. 1926.
[6] Burns, R. K., Jr.: *Anat. Rec.* 71: 447. 1938.
[7] Burns, R. K., Jr.: *Anat. Rec.* 73: 73. 1939.

CURRENT ENDOCRINE LITERATURE

COLLABORATORS

Fuller Albright
Wayne J. Atwell
Geoffrey Bourne
Israel Bram
John C. Burch
H. J. Deuel, Jr.
John C. Donaldson
Maurice H. Friedman
Murray B. Gordon
Arthur Grollman
Frank A. Hartman
E. C. Hamblen
F. C. Koch
Edward Larson
J. T. Lewis
Joseph M. Looney
A. E. Meyer
Warren O. Nelson
J. P. Pratt
A. T. Rasmussen
Elmer L. Sevringhaus
L. B. Shpiner
D. L. Thomson
R. L. Zwemer

ADVANCE ABSTRACTS

AMERICAN CHEMICAL SOCIETY, DIVISION OF MEDICINAL CHEMISTRY. SYMPOSIUM ON ENDOCRINES. *To appear in this Journal.*

MASON, H. L. Chemistry of the adrenal cortical hormones.

This paper presents a summary of recent work on the chemistry of the adrenal cortical hormones. This includes material both on the crystalline steroid compounds and the water-soluble amorphous mixture which apparently contains the major portion of the activity.

GROLLMAN, A. The rôle of the adrenal glands in the animal economy.

A review of the various theories advanced to explain their function as well as recent work on the physiology and pathology of the adrenals is presented.

MACCORQUODALE, D. W. The chemistry of the sex hormones.

A detailed discussion of the chemistry of the sex hormones is presented.

NOVAK, E. Clinical employment of the female sex hormones.

This paper aims to epitomize the salient points in the treatment of disorders of the female reproductive cycle.

JENSEN, H., AND S. TOLKSDORFF. The relationship of the anterior pituitary to sex and metabolism.

This paper is a discussion of the relations of the anterior pituitary to sex, to the adrenals and thyroid; the influence of the anterior pituitary on growth; prolactin and the relationship of the anterior pituitary to metabolism.

MIRSKY, I. A. Some aspects of the biology of the pancreatic hormones.

The various biochemical and physiological aspects of the pancreas with regard to insulin are discussed.

ABRAMOWITZ, A. A., AND F. L. HISAW. The effects of proteolytic enzymes on purified gonadotropic hormones. *To appear in this Journal.*

Crystalline trypsin at PH 7.1 completely destroys FSH, LH and PU. Crystalline chymotrypsin at PH 7.6 destroys PU completely, but only partly inactivates FSH and LH. Crude ptylin at PH 7.1 destroys FSH and PU slowly, but does not inactivate LH. Commercial papain at PH 7.1 destroys PU completely, and FSH partly, but does not affect LH.

CLAUSEN, F. W., AND C. B. FREUDENBERGER. A comparison of the effects of male and female sex hormones on immature female rats. *To appear in this Journal.*

Sixty immature female Wistar albino rats were divided into 4 groups of 15 animals each. The groups, injected subcutaneously every other day, received the following doses at each injection: 400 I.U. (120 R.U.) of theelin in oil; 1 mg. of testosterone propionate (oreton); and the same amounts of the two hormones combined. The controls received 0.2 cc. of olive oil. The animals received the first injection on the 21st day of age, and the last, on the 29th day of age. They were killed on the 31st day of life. The size of the uterus was increased while that of the thymus was decreased by the treatment. Theelin and testosterone propionate combined produced the greatest change. Testosterone propionate produced a greater

change than theelin. The pituitary weight was increased while the adrenal weight was decreased by theelin, but not by testosterone propionate or the two hormones together. No significant changes were produced in the weights of the ovaries and head, or in the body length. The thyroid weight was increased in all of the test groups, though not significantly. The both-hormone group had the largest glands. Body weight gain was significantly enhanced by testosterone proprionate. Theelin decreased body weight gain, though not significantly so in this investigation. The combination of the hormones made the body weight gain parallel that of the control series. Histologically, the ovaries of the testosterone propionate and both-hormone groups contained the largest follicles. No corpora lutea were found in any instance. The vaginae were prematurely opened by the hormones. Antagonistic action between the two hormones was evidenced by changes produced in the body weight, hypophysis, and adrenals.

Dow, D., and S. Zuckerman. Further observations on the vaginal pH of monkeys treated with estrogenic substances. *To appear in this Journal.*

Day to day variations in the vaginal pH of 12 gonadectomized rhesus monkeys were determined by the glass electrode method during experiments in which the animals were being injected with either estrone, estradiol, diethyl-stilbestrol or estrogenic hormone followed by progesterone. Observations were made during 41 experiments. It was confirmed that the pH falls sharply when animals are under the influence of estrogenic stimulation and it was found that this effect is demonstrated as markedly by diethyl-stilbestrol as by natural estrogenic substances. It was also observed that the vaginal pH rises rapidly when injections of progesterone are given immediately after a course of daily injections of estrogenic hormone and that phases of uterine bleeding are generally preceded by a rise in the vaginal pH. The same degree of estrogenic stimulation is not necessarily associated with the same pH in different animals or in the same animals at different times.

Hasler, A. D., R. K. Meyer and H. M. Field. Spawning induced prematurely in trout with the aid of pituitary glands of the ovary. *To appear in this Journal.*

Both mature eggs and sperm were obtained from rainbow trout 6 to 7 weeks in advance of the onset of the normal spawning period in Wisconsin by intraperitoneal injections of fresh or acetone-dried pituitary glands of the carp. Pregnant mare serum and the follicle stimulating fraction prepared from sheep pituitary failed to induce spawning prematurely in trout.

Holden, R. B. Vascular reactions of the uterus of the immature rat. *To appear in this Journal.*

The vascular responses of the uterus of the immature rat have been studied by the intravenous injection of mercuric sulfide in vivo and by direct microscopic observation of living vessels transilluminated by means of a Lucite rod. Following a single injection of 0.1 r estradiol there results a uterine hyperemia, the development of which is shown to parallel closely the early increase in weight and water content of the uterus likewise induced by estrin. Various drugs were tested for their vasomotor effects. From these it was evident that these estrin effects on the vessels were not mediated by acetyl choline. Evidence is given that the mediator may be an H-substance. Pituitrin, epinephrine and choline derivatives cause vasoconstriction. Eserine, atropine and yohimbine had no vasomotor effect. Padutin and histamine produce vasodilatation.

Katzman, P. A., N. J. Wade and E. A. Doisy. Progonadotropic sera of animals treated with hypophysial extracts. *To appear in this Journal.*

In previous publications we reported that the chronic treatment of adult, female rats with implants or extracts of rats' hypophyses failed to produce a refractory state in the treated animals. On the contrary, the serum of such animals was found to enhance the action of the gonadotropic material of rats' anterior pituitary in immature female rats. The present report consists of an extension of our work dealing with the chronic treatment of animals with homozoic pituitary extracts and a chemical study of the nature of the augmentative substance found in the serum. Three sheep, two ewes and one ram, have been injected daily for 6 to 7 months, to date, with an extract of the pituitary glands of sheep. Two hogs, a boar and an immature female, have received an extract of hog's pituitaries for one year and 20 weeks, respectively. The serum of the sheep has in no case developed inhibitory properties toward the gonadotropic substance of the injected extract as tested in immature female rats. In each case, on the other hand, after a variable latent period, the serum developed augmentative properties which have persisted in spite of continued treatment. The serum of the boar has failed to elicit definite enhancing or inhibitory responses when tested against the extract of hogs' pituitaries. The female has not been treated long enough to justify any conclusions at the present time. The augmenting action of the sheep's serum appears to be rather highly specific. It exerted no influence on the gonadotropic substances of the pituitaries of hogs and beef, or the urines of pregnant and castrated women but it augmented the action of extracts of rats' hypophyses. The serum may be administered subcutaneously or intraperitoneally. We prefer the latter route because it eliminates the possibility of the serum mixing with the subcutaneously injected pituitary extract.

The gonadotropic response of intraperitoneally injected sheep pituitary extract is also augmented by subcutaneously injected serum. When administered alone, the serum has no observable effect on the reproductive organs of immature and mature female rats or upon virgin female rabbits. This suggests that the augmenting substance does not act by stimulating the pituitary of the test animal to produce more gonadotropic hormone. Fractionation of the serum with Na_2SO_4 has resulted uniformly in practically complete precipitation of the augmentative activity with the euglobulin fraction. Relatively little activity has been demonstrated in the pseudoglobulin precipitate. Solutions of the active substance are quite stable if they are stored in the frozen state. A stable powder may also be prepared by drying the euglobulin precipitate in a vacuum desiccator. The activity is readily adsorbed from a solution of the euglobulin fraction on alumina gel at a slightly acid pH and is effectively freed from the alumina by alkaline phosphate solution. It does not dialyze through Visking membrane but a portion of the activity remains in solution following the complete precipitation of the euglobulin by this means.

Sprunt, D. H. The part played by age and the female sex hormones in resistance to infection. *To appear in this Journal.*

The literature in regard to the effect of age and the female sex hormones on the response of the host to disease agents is reviewed. It is shown that this literature may be divided into 3 groups of papers: first, those on the effect on antibody formation; second, on morphological changes; and third, on the dispersion and concentration of disease agents in the skin. It is shown that age increases the ability of the body to produce antibodies, that certain of the sex hormones can change the tissues so that resistance to infection is changed, that the dispersion or concentration of disease agents is affected by the endocrine pattern of the host, and that this concentration or dispersion affects the host's resistance to disease.

Stewart, H. L., Jr., and J. P. Pratt. Effect of prolactin on mammary gland secretion. *To appear in this Journal.*

Three hundred and eight consecutive nursing mothers were studied to determine what constitutes normal breast secretion in women. The average daily milk consumption of 64 breast-fed babies during the first 10 postpartum days is presented. Standards were established to measure the apparent action of prolactin on deficient lactation. It is shown that a secretion of 250 cc. or less of breast milk on the 5th postpartum day is a deficiency of lactation. Twenty-four cases of lactation deficiency were studied. Fourteen cases were administered 1000 U of prolactin daily from the 6th to the 10th postpartum days; 10 cases received a control solution. The prolactin cases showed no greater increase of milk secretion than the controls. Similar observations were made on 10 mothers who secreted more than 250 cc. of milk on the 5th postpartum day but had insufficient milk for full-breast nursing. No injections were administered. A greater average increase of breast milk was noted in this group.

Talbot, N. B. The inactivation of endogenous estrogen by the liver. *To appear in this Journal.*

The evidence of in vitro experiments performed by other investigators suggests that the liver is the chief organ responsible for the inactivation of estrogen in the animal body. Intact 21-day-old female rats poisoned with a mixture of carbon tetrachloride and alcohol show signs of acute liver damage within 48 hours. At the time when the animals show the greatest prostration, their uteri are stimulated by an estrogenic substance as evidenced by increased weight, edema and congestion. Castration of the rats prior to the administration of the poison prevents the uterine reaction. From these facts the following conclusions are drawn. (*a*) The ovaries of 21 to 24-day-old rats normally secrete small amounts of estrogen. (*b*) In the normal intact animal, the liver inactivates most of the circulating estrogen and thereby keeps the concentration of the substance in the blood stream at a very low level. (*c*) When the liver is severely poisoned, it is no longer capable of inactivating estrogen efficiently. As a consequence, the concentration of estrogen in the circulation rises and the characteristic uterine response becomes evident.

Uotila, U. U. The rôle of the pituitary stalk in the regulation of the anterior pituitary, with special reference to the thyrotropic hormone. *To appear in this Journal.*

After pituitary stalk section in rats, enough thyrotropic hormone is produced to keep the thyroid histologically normal under ordinary conditions. However, stalk section, followed by exposure to cold, prevents the increased secretion of thyrotropic hormone and thyroid hypertrophy which normally occur in intact animals on exposure to cold. This negative effect is not due to a general pituitary insufficiency. Hence, it follows that in animals exposed to prolonged cold the hypothalamus stimulates the secretion of thyrotropic hormone by impulses transmitted through the pituitary stalk. The rôle of the pituitary stalk in this regulation is more important than that of the cervical sympathetics. After pituitary stalk section in rats, as after cervical sympathectomy, the adrenals remain normal and react normally to cold with hypertrophy. Corticotropic secretion is hence probably under humoral control. After pituitary stalk section in rats, the testicles and seminal vesicles remain normal, but the usual damaging effect of cold on spermatogenesis is abolished. Hence, the influence of temperature on the secretion of gonadotropic

hormones must be to a certain extent under hypothlamic control. Stalk section does not significantly affect the growth of rats. The anterior pituitary appears to have a basic secretory rhythm which is independent of the pituitary stalk under ordinary conditions. This rhythm is largely under humoral influences. Nevertheless, available evidence indicates that the hypothalamus and pituitary stalk participate in the regulation of anterior pituitary secretion in specific adjustments to certain environmental situations. In the present experiments, for example, evidence is adduced indicating the mediation of stimuli through the stalk upon prolonged exposure of the animal to cold.

VAN DYKE, H. B., AND J. S. CHEN. The assay of progesterone by the production of an artificial pregnancy-response of the feline uterus. *To appear in this Journal.*

The assay of progesterone by producing an artificial pregnancy-response of the uterus of the cat (contraction in response to sympathetic stimulation) was studied. A preliminary sensitizing dose of estrogen is of great importance; for this purpose 0.1 mg. of estradiol was usually injected. The dose of progesterone causing a pregnancy-reversal in 50% of animals is estimated to be 0.41 mg. in normal adult cats and 0.45 mg. in ovariectomized adults. At higher dose levels, normal cats are relatively more responsive; however, it appears that the uteri of about 25% of all cats respond poorly. The above account refers only to the detection of the response by the intravenous injection of adrenaline. Neither pregnadiol nor testosterone propionate causes a similar reversal in doses of 30 mg. or more. The response of the feline uterus to sympathetic stimulation appears not to be related to the histology of the endometrial glands or to the sensitivity of the uterus toward the oxytocic action of posterior-pituitary extract.

VIDGOFF, B., AND R. HILL. Studies on the inhibitory hormone of the testes. III. Histological effects on the male sex organs of the white rat. *To appear in this Journal.*

The histological effects of an extract from bull testes on the sex organs of the white rat are demonstrated by photomicrographs and description. The changes in the accessory sex organs are atrophic and degenerative in nature and simulate closely the picture seen in the prostate and seminal vesicle of the castrated rat. The changes in the testicle consist of interference with the process of spermatogenesis, atrophic and degenerative changes. The findings are discussed in the light of the inhibitory action of the extract on the anterior pituitary.

WOLFE, J. M., AND J. B. HAMILTON. Action of testosterone propionate on the structure of the anterior pituitary of the female rat with particular reference to the effects of prolonged administration on the levels of cells. *To appear in this Journal.*

Twenty-two immature female rats received daily injections of from 2 to 8 mg. of testosterone propionate daily for periods of 10 to 20 days. Sixteen litter-mate sisters were used as controls. The average pituitary weight of the injected rats was slightly less than that of the controls. It was found that in the injected rats degranulation among the basophiles occurred but noteworthy changes were not observed in the eosinophiles. The following experiments were carried out to determine the reaction of the anterior pituitary of the female rat to chronic administration of androgen. One or two females from a litter (approximately 60 days of age) received daily injection for 90 days. For each injected female an untreated brother and sister were used as controls. Four levels of injection were used: 1.0 mg., 0.5 mg., 0.25 mg., and 0.1 mg., all daily for 90 days. In each group there were 4 to 6 injected females and equal numbers of control brothers and sisters. Judged in relation to body weight, the weight of the pituitaries and adrenals of the injected females approached those of their control brothers and diverged from those of their control sisters. An increase in the levels of the eosinophiles and granular basophiles occurred in the anterior pituitaries of the injected females resulting in a cellular pattern which approached that found in their control brothers and widely diverged from that of the control sisters. This masculinizing effect was most marked in the females receiving the highest level of androgen. Cell counts were made and all quantitative data were handled by statistical methods.

ADRENALS

BLANCHARD, E. W., AND R. C. TALLMAN. The oral assay of adrenal cortical hormone. *Am. J. Physiol.* 124: 583. 1938.

A modified oral rat unit defined as "The amount of hormone material per day which will maintain life and normal growth for a period of 14 days in 80% of a group of not less than 10 bilaterally adrenalectomized rats, operated when approximately 50 gm. in weight and which die following the removal of the hormone" is proposed.—F. A. H.

CAHILL, G. F. The adrenogenital syndrome and adrenocortical tumors. *New England J. Med.* 218: 803. 1938.

The results of observations on 27 female and 8 male patients showing adrenocortical disturbances were reported. Of the male cases 4 were in children between the ages of 4 and 11, all of whom showed precocious maturity, but only 2 gave evidence of anatomic adrenal changes. The most frequently occurring change was that towards masculinization in the female, and this was found in 26 of the 27 cases. Only 6 of the cases had definitely proven adrenal tumors with adrenogenital syndrome and 1 case showed a tumor without endocrine symptoms. Operation was performed in 9 cases and of these 5 died. The results in the cases with carcinoma without hormonal symptoms were less favorable than in those showing definite adrenogenital signs. Treatment with female sex hormones in those cases without tumor did not prove of any particular value. The use of perineal air injections was helpful in visualizing adrenal abnormalities by x-ray. Some of the cases showed mental alterations which were very pronounced, and in one case more marked than the physical changes. An estimation of sex hormone excretion showed a marked increase in androgen output which decreased when the tumor was removed.—J. M. L.

CAMP, W. J. R. The identity of action of epinephrine and potassium. *J. Pharmacol. & Exper. Therap.* 66: 6. 1939.

Since the action of epinephrine and potassium is identical, the author suggests the administration of potassium to increase the effectiveness of epinephrine.—E. L.

DE WESSELOW, O. L. V. S., AND W. A. R. THOMSON. Sodium and Potassium in Addison's disease. *St. Thomas's Hosp. Rep.* 3: 28. 1938.

A study of 3 clinical cases is reported. All 3 showed sub-normal serum NaCl, the degree of deficit correlating with the severity of the symptoms. The Addisonian patients excreted as freely from the reduced blood level as normal subjects from a higher level hence a renal factor is suggested. Cortical extract was necessary to bring the NaCl up to normal. Attempts to this end with NaCl alone resulted, in 2 of the 3 cases, in edema. Although the blood K trend was high no correlation could be seen between K titre and severity of symptoms. A practicable low K dietary is described.—R. G. H.

GAUNT, R., AND H. W. HAYS. Rôle of progesterone and other hormones in survival of pseudo-pregnant adrenalectomized ferrets. *Am. J. Physiol.* 124: 767. 1938.

Estrus occurring spontaneously or induced by gonadotropic agents or esttrin increases the cortical hormone requirement in adrenal insufficiency in the ferret. Pseudopregnancy by luteinization of the ovaries makes cortical hormone unnecessary in adrenal insufficiency. This probably is due to the secretion of progesterone because 2 mg. per day of this substance will maintain adrenalectomized ferrets of either sex in excellent health. Spontaneous estrus is prevented by progesterone in the ferret. Adrenalectomized ferrets are benefitted by neither testosterone propionate nor AP extract which maintains the lives of adrenalectomized cats.—F. A. H.

GERSH, I. AND A. GROLLMAN. Kidney function in adrenal cortical insufficiency. *Am. J. Physiol.* 125: 66. 1939.

The authors failed to find any histological changes in the kidney (and liver) of rats, cats and dogs which could be attributed to adrenal cortical insufficiency. Preparations of the kidneys fixed in formalin—Zenker's fluid and sectioned at 5μ were stained with hematoxylin and eosin or with Mallory's triple stain. Histochemical studies (by means of ferrocyanide distribution investigations) in the early stages of adrenal cortical insufficiency demonstrated no clearly visible morphological or functional pathology. In advanced insufficiency the rate of absorption of water by the tubules was increased. In the terminal stages of insufficiency glomerular filtration was decreased or absent. No histological evidence that the neurohypophysis is stimulated in adrenal insufficiency was obtained.—Lena A. Lewis.

HAMPEL, E. Changes in the brain in a case of Addison's disease. *Ztschr. f. d. ges. Neurol. u. Psychiat.* 158: 91. 1937.

Hampel reports the case of a man aged 23 who had had Addison's disease for 8 years. He died in coma. Diffuse glial proliferation was observed in the white substance of the cerebral hemispheres. Demyelination was noted only in the periventricular region. The histopathologic picture suggests that of a toxic process associated with Addison's disease. There was no familial history of diffuse sclerosis.—Abstract from *Arch. Neurol. & Psychiat.*

INGLE, D. J. The time for the occurrence of cortico-adrenal hypertrophy in rats during continued work. *Am. J. Physiol.* 124: 627. 1938.

When rats were anesthetized and the gastrocnemius muscle made to lift 100 gm. 3 times per second a definite gain in the average weights of the adrenal glands was demonstrated to occur as early as 12 hours

after the beginning of work. This hypertrophy did not occur during work when similar rats were either hypophysectomized or treated with cortin. The administration of adrenotropic hormone to hypophysectomized rats likewise induced a hypertrophy of the adrenal glands by the end of 12 hours.—Author's Summary.

KENDALL, E. C., E. V. FLOCK, J. L. BOLLMAN AND F. C. MANN. The influence of cortin and sodium chloride on carbohydrate and mineral metabolism in adrenalectomized dogs. *J. Biol. Chem.* 126: 697. 1938.

The influence of continuous intravenous injections of 10% glucose with and without cortin and NaCl on the K levels of adrenalectomized and normal dogs was studied. In every instance the injections caused a decrease in serum K. If cortin or NaCl was added there was a mild diuresis without glycosuria but K was readily excreted. Glucose injected alone caused anuria. The blood sugar was high during the period of injection but fell afterwards to hypoglycemic levels. The decrease in active utilization of the sugar was followed by an increase in serum K, which was associated with symptoms of weakness and prostration in the adrenalectomized dogs that were deprived of cortin for 1 to 5 days. All of these animals developed anuria. Weakness and prostration did not occur if there had been a diuresis. Sodium chloride was as effective as cortin in preventing anuria. Only the concentration of the K could be correlated with cortico-adrenal deficiency. The phosphate levels changed with the concentration of K but no consistent changes were found in the Na or Cl levels, the osmotic pressure or hematocrit values. —J. M. L.

LEWIS, J. T., AND J. M. BARMAN. Effects of prolonged treatment with adrenalin and blood from the adrenal vein (Efectos del tratamiento prolongado con adrenalina y sangre de la vena suprarrenal). *Rev. Soc. Argent. de Biol.* 14: 561. 1938.

Prolonged treatment with intravenous injection of adrenalin (1 mg. daily for several months) did not alter the capacity of response to adrenalin as measured by the rise in blood pressure in dogs. No alterations were observed in the microscopic study of the tissues, the arteries (large and small) were normal. Intravenous injections of adrenal blood with adrenalin effect, from dog to dog, repeated at intervals for several weeks did not alter the blood pressure response to adrenalin or to adrenal blood. Interaperitoneal injections of adrenal blood with adrenalin effect from dogs into rabbits did not alter the response of these last to the blood pressure effect of adrenalin or adrenal blood of dog. The rabbits were sensitive to dog's blood and frequently died of anaphylactic shock. An adrenalin antihormone was therefore not made evident.—J. T. L.

LUCO, J. V. The defatiguing effect of adrenaline. *Am. J. Physiol.* 125: 196. 1939.

The defatiguing action of adrenaline was studied in cats, on normal muscles stimulated indirectly and on denervated muscles stimulated directly. Instead of this defatiguing effect a depression sometimes occurred when injections were made without ergotoxine. After ergotoxine no depressions were encountered. The depression without ergotoxine is interpreted as due to vasoconstriction. In non-fatigued normal muscles adrenaline may, however, produce a decrease of tension when administered after prostigmin. This effect is not vascular because it appears after ergotoxine. Adrenaline increases the responses of denervated muscles to acetylcholine. Ergotoxine favors this action.—From Author's Summary.

LUDUEÑA, F. P. Action of cocaine and pyrogallol on the retractor penis (Sobre la famacología del sistema nervioso autónomo pelviano. Acción de la cocaína y del pirogalol sobre el músculo retractor del pene). *Rev. Soc. Argent. de Biol.* 14: 535. 1938.

Experiments were carried out on chloralosed dogs, registering the contraction of the retractor penis in situ. The minimum dose of adrenalin chlorhydrate that given intravenously produced contraction varied from 0.1 to 1 mg. in different animals. Cocaine in doses of 0.5 to 3 mg. per kg. sensitized the muscle to adrenalin and produced a small increase in the action of the sympathetic. Pyrogallol (20 to 40 mg. per kg.) did not modify the tone of the retractor penis; it did not modify the height of the contraction produced by adrenalin, but increased its duration in the majority of the experiments. Simultaneous administration of cocaine and pyrogallol greatly increased the effect of adrenalin. Cocaine in doses of 1 to 5 mg. per kg. produced intense contraction of the retractor penis, by a peripheral mechanism. Pyrogallol potentiates this effect of cocaine. Piperidin-methyl-3-benzodioxane (F. 933), suppressed the effect of cocaine on the retractor penis, but did not modify the contraction produced by ergotoxin (musculotropic action). Cocaine in concentrations of 1:50,000 to 1:100,000 increased the effect of adrenalin on the isolated retractor penis.—J. T. L.